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(54) Title: TRANSGENIC EXPRESSION CASSETTES FOR THE EXPRESSION OF NUCLEIC ACIDS IN PLANT BLOOMS
(54) Bezeichnung: TRANSGENIC EXPRESSIONSKASSETTEN ZUR EXPRESSION VON NUKLEINSÄUREN IN DER
PFLANZLICHEN BLÜTE

(57) Abstract: The invention relates to methods for the targeted, transgenic expression of nucleic acid sequences in plant blooms and
transgenic expression cassettes and expression vectors which have promoters with an expression specificity for plant blooms. The
invention further relates to organisms modified with said transgenic expression cassettes or expression vectors (preferably plants),
cultures derived therefrom, parts or propagation material and the use of the above for the production of human and animal foodstuffs,
seed stock, pharmaceuticals or fine chemicals.

(57) Zusammenfassung: Die Erfindung betrifft Verfahren zur gezielten, transgenen Expression von Nukleinsäuresequenzen in der
pflanzlichen Blüte, sowie transgene Expressionskassetten und Expressionsvektoren, die Promotoren mit einer Expressionspezifität
für die pflanzliche Blüte enthalten. Die Erfindung betrifft ferner mit diesen transgenen Expressionskassetten oder Expressionsvektoren
modifizierte Organismen (bevorzugt Pflanzen), davon abgeleitete Kulturen, Teile oder Vermehrungsmaterial, sowie die Verwendung
dieser zur Herstellung von Nahrungs-, Futtermittel-, Saatgut-, Pharmazeutika oder Feinchemikalien.

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(54) **CASSETTES D'EXPRESSION TRANSGENIQUE POUR L'EXPRESSION D'ACIDES NUCLEIQUES DANS UNE
FLEUR VEGETALE**

(54) **TRANSGENIC EXPRESSION CASSETTES FOR THE EXPRESSION OF NUCLEIC ACIDS IN PLANT BLOOMS**

(57)

The invention relates to methods for the targeted, transgenic expression of nucleic acid sequences in plant blooms and transgenic expression cassettes and expression vectors which have promoters with an expression specificity for plant blooms. The invention further relates to organisms modified with said transgenic expression cassettes or expression vectors (preferably plants), cultures derived therefrom, parts or propagation material and the use of the above for the production of human and animal feedstuffs, seed stock, pharmaceuticals or fine chemicals.



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(54) Title: TRANSGENIC EXPRESSION CASSETTES FOR THE EXPRESSION OF NUCLEIC ACIDS IN PLANT
BLOOMS

(57) Abrégé/Abstract:

The invention relates to methods for the targeted, transgenic expression of nucleic acid sequences in plant blooms and transgenic expression cassettes and expression vectors which have promoters with an expression specificity for plant blooms. The invention further relates to organisms modified with said transgenic expression cassettes or expression vectors (preferably plants), cultures derived therefrom, parts or propagation material and the use of the above for the production of human and animal feedstuffs, seed stock, pharmaceuticals or fine chemicals.

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(57) Abstract: The invention relates to methods for the targeted, transgenic expression of nucleic acid sequences in plant blooms and transgenic expression cassettes and expression vectors which have promoters with an expression specificity for plant blooms. The invention further relates to organisms modified with said transgenic expression cassettes or expression vectors (preferably plants), cultures derived therefrom, parts or propagation material and the use of the above for the production of human and animal feedstuffs, seed stock, pharmaceuticals or fine chemicals.

(57) Zusammenfassung: Die Erfindung betrifft Verfahren zur gezielten, transgenen Expression von Nukleinsäuresequenzen in der pflanzlichen Blüte, sowie transgene Expressionskassetten und Expressionsvektoren, die Promotoren mit einer Expressionsspezifität für die pflanzliche Blüte enthalten. Die Erfindung betrifft ferner mit diesen transgenen Expressionskassetten oder Expressionsvektoren transformierte Organismen (bevorzugt Pflanzen), davon abgeleitete Kulturen, Teile oder Vermehrungsgut, sowie die Verwendung derselben zur Herstellung von Nahrungs-, Futtermitteln, Saatgut, Pharmazeutika oder Feinchemikalien.

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TRANSGENIC EXPRESSION CASSETTES FOR THE EXPRESSION OF NUCLEIC
ACIDS IN PLANT BLOOMS

10 The invention relates to methods for the targeted transgenic expression of nucleic acid sequences in the flower of plants, and to transgenic expression cassettes and expression vectors which comprise promoters having an expression specificity for the flower of plants. The invention further relates to organisms (preferably plants) transformed with these transgenic expression cassettes or expression vectors, to cultures, parts or propagation material derived therefrom, and to the use of the same for producing human and animal foods, seeds, pharmaceuticals or fine chemicals.

20 The aim of biotechnological operations on plants is to produce plants with advantageous novel properties, for example for increasing the agricultural productivity, for increasing the quality of human foods or for producing particular chemicals or pharmaceuticals (Dunwell JM (2000) J Exp Bot 51 Spec No:487-96). A basic precondition for transgenic expression of particular genes is the provision of promoters which are functional in plants. Promoters are important tools in plant biotechnology for controlling the expression of particular genes in a transgenic plant and thus achieving particular traits of the plant.

30 Various promoters functional in plants are known, for example constitutive promoters such as the promoter of the agrobacterium nopaline synthase, the TR double promoter or the promoter of the cauliflower mosaic virus (CaMV) 35S transcript (Odell et al. (1985) Nature 313:810-812). A disadvantage of these promoters is that they are constitutively active in virtually all tissues of the plant. Targeted expression of genes in particular plant parts or at particular times of development is not possible with these promoters. There is thus a particularly great need for promoters having a defined activity profile and a specificity for particular plant tissues.

Promoters having specificities for various plant tissues such as anthers, ovaries, flowers, leaves, stalks, roots, tubers or seeds have been described. The stringency of the specificity and the expression activity of these promoters varies widely.

40 The flower of plants serves for sexual reproduction of flowering plants. The flowers of plants - especially the petals - frequently accumulate large amounts of secondary plant products such as, for example, terpenes, anthocyanins, carotenoids,

alkaloids and phenylpropanoids, which serve as scents, defensive substances or as colorants in the flower. Many of these substances are of commercial interest. In addition, the flower bud and the flower of the plant is a sensitive organ, especially to stress factors such as cold.

Flower-specific promoters such as, for example, the phytoene synthase promoter (WO 92/16635), the promoter of the P-rr gene (WO 98/22593) or the promoter of the APETALA3 gene (Hill TA et al. (1998) Development 125:1711-1721) are known. However, all these promoters have one or more disadvantages which are prejudicial to wide use:

- 1) within the flower they are specific for one or more flower tissues and do not guarantee expression in all tissues of the flower.
- 2) they are -- as in the example of the APETALA3 gene which is involved in flower development - highly regulated during flower development and are not active in all phases of flower development.
- 3) they occasionally show strong secondary activities in other plant tissues.

Despite the large number of known plant promoters, there is a need for promoters having a specificity for the flower of plants and guaranteeing high expression over a long period of flower development and flowering.

It is an object of the present invention to provide methods and suitable promoters for the targeted transgenic expression of nucleic acids in flower tissues.

We have found that this object is achieved by providing promoters of ϵ -cyclase. These promoters show an usually strong expression in numerous flower organs.

A first aspect of the invention relates to methods for the targeted transgenic expression of nucleic acid sequences in the flower of plants, including the following steps

- I. introduction of a transgenic expression cassette into plant cells, where the transgenic expression cassette comprises at least the following elements

- a) at least one promoter sequence of a gene coding for an ϵ -cyclase, and
- b) at least one further nucleic acid sequence, and
- c) where appropriate further genetic control elements,

10 where at least one of said promoter sequences and one further nucleic acid sequence are functionally linked together, and the further nucleic acid sequence is heterologous in relation to the promoter sequence or the plant cell, and

II. selection of transgenic cells which comprise said expression cassette stably integrated into the genome, and

III. regeneration of complete plants from said transgenic cells, where at least one of the further nucleic acid sequences is expressed in the flower.

20 A further aspect relates to transgenic expression cassettes as can be employed in the method of the invention. The transgenic expression cassettes preferably include for the targeted transgenic expression of nucleic acid sequences in the flower of plants

- a) at least one promoter sequence of gene coding for an ϵ -cyclase, and
- b) at least one further nucleic acid sequence, and
- c) where appropriate further genetic control elements,

30 where at least one promoter sequence and one further nucleic acid sequence are functionally linked together, and the further nucleic acid sequence is heterologous in relation to the promoter sequence.

In a preferred embodiment of the method of the invention and/or of the expression cassettes of the invention, "promoter sequence of a gene coding for an ϵ -cyclase" means a sequence selected from the group of sequences consisting of

- i) the promoter sequence of the ϵ -cyclase from *Tagetes erecta* as shown in SEQ ID NO: 1, the ϵ -cyclase from *Arabidopsis thaliana* as shown in SEQ ID NO: 7, the ϵ -cyclase from *Oryza*
- 40

sativa as shown in SEQ ID NO: 8, and

- 10 ii) functional equivalents of the promoter sequences as shown in SEQ ID NO: 1, 7 or 8 having substantially the same promoter activity as the promoter of the ϵ -cyclases as shown in SEQ ID NO: 1, 7 or 8 and
- 10 iii) functionally equivalent fragments of the sequences under i) or ii) having substantially the same promoter activity as the promoter of ϵ -cyclases as shown in SEQ ID NO: 1, 7 or 8.

It is particularly preferred for "promoter sequence of a gene coding for an ϵ -cyclase" to mean the promoter sequence from *Tagetes erecta* as shown in SEQ ID NO: 1 and functionally equivalent fragments thereof.

The expression cassettes of the invention may comprise further genetic control sequences and/or additional functional elements.

- 20 It is possible and preferred for the transgenic expression cassettes to make possible, through the nucleic acid sequence to be expressed transgenically, the expression of a protein encoded by said nucleic acid sequence and/or the expression of a sense-RNA, antisense-RNA or double-stranded RNA encoded by said nucleic acid sequence.

A further aspect of the invention relates to transgenic expression vectors which comprise one of the expression cassettes of the invention.

- 30 A further aspect of the invention relates to transgenic organisms which comprise one of the expression cassettes or expression vectors of the invention. The organism can be selected from the group consisting of bacteria, yeasts, fungi, nonhuman animals and plant organisms or of cells, cell cultures, parts, tissues, organs or propagation material derived therefrom, and the organism is preferably selected from the group of agricultural crop plants.

A further aspect of the invention therefore relates to an isolated nucleic acid sequence including the promoter of the ϵ -cyclase from *Tagetes erecta* as shown in SEQ ID NO: 1, and functionally equivalent fragments thereof.

In a preferred embodiment, the nucleic acid sequence of the invention or the transgenic expression cassette of the invention

in the form of a functionally equivalent promoter sequence includes, besides the sequence shown in SEQ ID NO: 1, additionally the sequence coding for the 5'-untranslated region of the ϵ -cyclase gene from *Tagetes erecta*. The sequence described by SEQ ID NO: 2 is particularly preferred.

10 In a further preferred embodiment, the nucleic acid sequence of the invention or the transgenic expression cassette of the invention in the form of a functionally equivalent promoter sequence includes, besides the sequence shown in SEQ ID NO: 1, additionally the sequence coding for the 5'-untranslated region of the ϵ -cyclase gene from *Tagetes erecta* and a sequence coding for a transit peptide, preferably for the transit peptide of the ϵ -cyclase protein from *Tagetes erecta* as shown in SEQ ID NO: 4. This sequence is preferably oriented in the 3' direction in relation to one of the promoters of the invention. The sequence described by SEQ ID NO: 3 is particularly preferred as promoter sequence in this connection.

20 A further aspect relates to the use of the isolated nucleic acid sequences, transgenic expression vectors or transgenic organisms of the invention for the transgenic expression of nucleic acids and/or proteins.

A further aspect of the invention relates to the use of the nucleic acid sequence of the invention for reducing the expression of an ϵ -cyclase. Included within this according to the invention are expression cassettes able to express a double-stranded RNA corresponding to the promoter sequence.

30 It is particularly preferred to use said transgenic organisms or cells, cell cultures, parts, tissues, organs or propagation material derived therefrom to produce human and animal foods, seeds, pharmaceuticals or fine chemicals, where the fine chemicals are preferably enzymes, vitamins, amino acids, sugars, saturated or unsaturated fatty acids, natural or synthetic flavorings, aromatizing substances or colorants. The invention further includes methods for producing said human and animal foods, seeds, pharmaceuticals or fine chemicals employing the transgenic organisms of the invention or cells, cell cultures, parts, tissues, organs or propagation material derived therefrom.

The transgenic expression cassettes of the invention are particularly advantageous for the following reason:

- a) they impart selective expression in the flower of plant and make numerous applications possible, such as, for example, resistance to stress factors such as cold or targeted synthesis of secondary plant products. Expression takes place throughout the period of flower development with high activity.

10 "Expression" means the transcription of the nucleic acid sequence which is to be expressed transgenically, but may also include - in the case of an open reading frame in the sense orientation - the translation of the transcribed RNA of the nucleic acid sequence to be expressed transgenically into a corresponding polypeptide.

"Transgenic" means - for example in relation to a transgenic expression cassette, a transgenic expression vector, a transgenic organism or methods for the transgenic expression of nucleic acids - all constructions resulting from methods of genetic manipulation, or methods using such, in which either

- 20 a) an ϵ -cyclase promoter (e.g. as shown in SEQ ID NO: 1, 7 or 8) or a functional equivalent thereof or a functionally equivalent fragment of the aforementioned, or
- b) the nucleic acid sequence which is to be transgenically expressed, are functionally linked to a promoter according to a), or
- c) (a) and (b)

30 are not located in their natural genetic environment or have been modified by methods of genetic manipulation, where the modifications may be for example substitutions, additions, deletions, inversions or inserts of one or more nucleotide residues. The promoter sequence of the invention (e.g. the sequence as shown in SEQ ID NO: 1, 7 or 8) contained in the expression cassettes is preferably heterologous in relation to the further nucleic acid sequence which is to be expressed transgenically and is functionally linked thereto.

"Heterologous" means in this connection that the further nucleic acid sequence does not code for the gene which is naturally under the control of said promoter.

"Natural genetic environment" means the natural chromosomal locus in the original organism or the presence in a genomic library. In the case of a genomic library, the natural genetic

environment of the nucleic acid sequence is preferably still retained at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, particularly preferably at least 1000 bp, very particularly preferably at least 5000 bp. A naturally occurring expression constant - for example the naturally occurring combination of the promoter as shown in SEQ ID NO: 1 and of a gene coding for a protein as shown in SEQ ID NO: 10 or 12 - becomes a transgenic expression construct when the latter is modified by unnatural, synthetic ("artificial") methods such as, for example, an in vitro mutagenesis. Appropriate methods are described (US 5,565,350; WO 00/15815; see also above).

"Transgenic" means in relation to an expression ("transgenic expression") preferably all expressions caused by use of a transgenic expression cassette, transgenic expression vector or transgenic organism - complying with the definitions given above.

The transgenic expression cassettes of the invention, and the transgenic expression vectors and transgenic organisms derived therefrom may include functional equivalents to the ϵ -cyclase promoter sequence described in SEQ ID NO: 1, 7 or 8.

Functional equivalents also include all the sequences derived from the complementary strand of the sequences defined by SEQ ID NO: 1, 7 or 8 and having substantially the same promoter activity. Particularly preferably included are the sequences shown in SEQ ID NO: 2 or 3, which, besides the promoter sequence, comprise the 5'-untranslated region or the 5'-untranslated region and the region coding for the transit peptide of the ϵ -cyclase from *Tagetes erecta*.

Functional equivalents means in particular natural or artificial mutations of the ϵ -cyclase promoter sequence described in SEQ ID NO: 1, 7 or 8, and the homologs thereof from other plant genera and species which still have substantially the same promoter activity as the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8.

A promoter activity is referred to as substantially the same when the transcription of a particular gene to be expressed is, under the control of, for example, a functional equivalent of the ϵ -cyclase promoter sequence described by SEQ ID NO: 1, 7 or 8, or of a functionally equivalent fragment thereof - under conditions

which are otherwise unchanged - higher in at least one flower tissue than in another non-flower tissue, for example the root or the leaves. In this connection, the expression under the control of one of the promoters of the invention in a flower tissue is preferably at least twice or five times, very particularly preferably at least ten times or fifty times, most preferably at least hundred times, that in another non-flower tissue, for example the root or the leaves.

- 10 "Flower" generally means a shoot of limited growth whose leaves have been transformed into reproductive organs. The flower consists of various "flower tissues" such as, for example, the sepals, the petals, the stamens or the carpels. Androecium is the term used for the totality of stamens in the flower. The stamens are located within the circle of petals and sepals. A stamen is composed of a filament and of an anther located at the end. The latter in turn is divided into two thecae which are connected together by a connective. Each theca consists of two pollen sacs in which the pollen is formed.

- 20 "Targeted" means in relation to expression in the flowers of plants preferably that the expression under the control of one of the promoters of the invention in at least one plant flower tissue is at least ten times, particularly preferably at least fifty times, very particularly preferably at least one hundred times that in a non-flower tissue such as, for example, the leaves.

- The sequences preferably employed for estimating the level of expression are those which code for easily quantifiable proteins. Very particular preference is given in this connection to reporter proteins (Schenborn E, Groskreutz D. (1999) Mol Biotechnol 13(1): 29-44) such as the green fluorescent protein (GFP) (Chiu WL et al. (1996) Curr Biol 6:325-330; Leffel SM et al. (1997) Biotechniques 23(5):912-8), chloramphenicol acetyltransferase, luciferase (Millar et al. (1992) Plant Mol Biol Rep 10:324-414), β -glucuronidase or β -galactosidase. Very particular preference is given to β -glucuronidase (Jefferson et al. (1987) EMBO J 6:3901-3907).
- 30

- "Conditions which are otherwise unchanged" means that the expression initiated by one of the transgenic expression cassettes to be compared is not modified by combination with additional genetic control sequences, for example enhancer sequences. Unchanged conditions further means that all general conditions such as, for example, plant species, stage of
- 40

development of the plants, culture conditions, assay conditions (such as buffer, temperature, substrates etc.) are kept identical between the expressions to be compared.

Functional equivalents of the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8 preferably includes sequences which

- a) have substantially the same promoter activity as the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8, and
- b) have a homology of at least 50%, preferably 70%, more preferably at least 80%, particularly preferably at least 90%, very particularly preferably at least 95%, most preferably 99%, with the sequence of the ϵ -cyclase promoter shown in SEQ ID NO: 1, 7 or 8, where the homology extends over a length of at least 100 base pairs, preferably at least 200 base pairs, particularly preferably of at least 300 base pairs, very particularly preferably of at least 400 base pairs, most preferably of at least 500 base pairs.

It is possible in this connection for the level of expression of the functional equivalents to differ both downwards and upwards from a comparison value. Preference is given in this connection to the sequences whose level of expression, measured on the basis of the transcribed mRNA or the subsequently translated protein, under conditions which are otherwise unchanged differs quantitatively by not more than 50%, preferably 25%, particularly preferably 10%, from a comparison value obtained with the promoters described by SEQ ID NO: 1, 7 or 8. Particularly preferred sequences are those whose level of expression, measured on the basis of the transcribed mRNA or the subsequently translated protein, under conditions which are otherwise unchanged exceeds quantitatively by more than 50%, preferably 100%, particularly preferably 500%, very particularly preferably 1000%, a comparison value obtained with the promoter described by SEQ ID NO: 1, 7 or 8.

Further examples of functionally equivalent promoter sequences employed in the transgenic expression cassettes or transgenic expression vectors of the invention can easily be found for example in various organisms whose genomic sequence is at least partly known, such as, for example, *Arabidopsis thaliana*, *Brassica napus*, *Nicotiana tabacum*, *Solanum tuberosum*, *Helianthus annuus*, *Linum sativum* oder *Oryza sativa*, followed by homology comparisons in databases. A possible and preferred starting point for this is the coding regions of the gene whose promoter is

described by SEQ ID NO: 1, 7 or 8. Starting from, for example, the cDNA sequences of these genes described by SEQ ID NO: 9, 11, 13 or 15 or the protein sequence derived therefrom and described by SEQ ID NO: 10, 12, 14 or 16 it is possible easily to identify, in a manner familiar to the skilled worker, the corresponding homologous genes - and thus the relevant promoter regions, in other plant species by screening databases or gene libraries (using appropriate gene probes).

- 10 In a further preferred embodiment, functional equivalents to the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8 include sequences which are located in a plant organism in the 5' direction in front of a genomic sequence which codes for an ϵ -cyclase.

ϵ -Cyclase means in general all proteins which have an ϵ -cyclase activity.

By ϵ -cyclase activity is meant the enzymic activity of an ϵ -cyclase.

- 20 An ϵ -cyclase means a protein which has the enzymatic activity of converting a terminal linear lycopene residue into an ϵ -ionone ring.

In particular, ϵ -cyclase means in general all proteins able to catalyze the cyclization of lycopene to δ -carotene (and where appropriate further to ϵ -carotene) and/or of neurosporene to α -zeacarotene. The ϵ -cyclase preferably has an oxidoreductase activity and/or naturally shows a predominant localization in the plastids, especially the chloroplasts and chromoplasts.

- 30 An ϵ -cyclase preferably means a protein having the enzymatic activity for converting lycopene into δ -carotene. Accordingly, ϵ -cyclase activity means the amount of lycopene converted by the ϵ -cyclase protein, or the amount of δ -carotene formed, in a particular time.

The ϵ -cyclase activity in genetically modified plants of the invention and in wild-type or reference plants is preferably determined under the following conditions:

the ϵ -cyclase activity can be determined by the method of Fraser and Sandmann (Biochem. Biophys. Res. Comm. 185(1) (1992) 9-15) in vitro if potassium phosphate as buffer (pH 7.6), lycopene as

substrate, stromal protein of paprika, NADP⁺, NADPH and ATP are added to a defined amount of plant extract.

The ϵ -cyclase activity in genetically modified plants of the invention and in wild-type and reference plants is particularly preferably determined by the method of Bouvier, d'Harlingue and Camara (Arch Biochem Biophys 346(1) (1997) 53-64): the in vitro assay is carried out in a volume of 0.25 ml. The mixture contains 50 μ M potassium phosphate (pH 7.6), various amounts of plant extract, 20 nM lycopene, 0.25 mg of paprika chromoplastid stromal protein, 0.2 μ M NADP⁺, 0.2 μ M NADPH and 1 μ M ATP. NADP/NADPH and ATP are dissolved in 0.01 ml of ethanol with 1 mg of Tween 80 immediately before addition to the incubation medium. After a reaction time of 60 minutes at 30°C, the reaction is stopped by adding chloroform/methanol (2:1). The reaction products extracted into chloroform are analyzed by HPLC.

An alternative assay with radioactive substrate is described in Fraser and Sandmann (Biochem Biophys Res Comm 185(1) (1992) 9-15). A further analytical method is described in Beyer, Kröncke and Nievelstein (J Biol Chem 266(26) (1991) 17072-17078).

20 In a preferred embodiment of the invention, functional equivalents of the ϵ -cyclase promoter described by SEQ ID NO: 1, 7 or 8 include all promoters which are located in a plant organism in the 5' direction in front of a genomic sequence which codes for an ϵ -cyclase having a homology of at least 60%, preferably at least 80%, particularly preferably at least 90%, most preferably at least 95%, with a protein as shown in SEQ ID NO: 10, 12, 14 or 16, where said promoters represent the natural promoter of said genomic sequence.

30 Functional equivalents of ϵ -cyclase promoter described by SEQ ID NO: 1, 7 or 8 particularly preferably include all promoters which are located in a plant organism in the 5' direction in front of a genomic sequence which codes for a nucleic acid sequence whose derived cDNA has a homology of at least 60%, preferably at least 80%, particularly preferably at least 90%, most preferably at least 95%, with the nucleic acid sequence as shown in SEQ ID NO: 9, 11, 13 or 15, where said promoters represent the natural promoter of said genomic sequence, and the cDNA codes for an ϵ -cyclase.

40 Preferred promoters include a sequence region of least 250 base pairs, preferably at least 500 base pairs, particularly

preferably 1000 base pairs, most preferably at least 2000 base pairs, in the 5' direction calculated from the ATG start codon of said genomic sequences.

Functional equivalents of the ϵ -cyclase promoter described by SEQ ID NO: 1, 7 or 8 are particularly preferably all promoters which are located in a plant organism in the 5' direction in front of a genomic sequence which codes for an ϵ -cyclase which comprises at least one of the following sequence motifs:

- | | | |
|----|---------------------------------|-----------------|
| | 1. G(G/C)GPAGL(A/S) (V/L)A | (SEQ ID NO: 17) |
| 10 | 2. (L/I) (N/G/S)RXYG(K/R) (V/L) | (SEQ ID NO: 18) |
| | 3. MVFMD(Y/W)RD | (SEQ ID NO: 19) |
| | 4. PTFLY(A/V)M(P/A) | (SEQ ID NO: 20) |
| | 5. AXMVHP(S/A)TGY(M/S)V(A/V)R | (SEQ ID NO: 21) |
| | 6. LWPXER(R/K)RQRXFF | (SEQ ID NO: 22) |

Very particularly preferred functional equivalents of the promoter described by SEQ ID NO: 1, 7 or 8 are those promoters which are located in a plant organism in the 5' direction in front of a genomic sequence which codes for a protein, where said
20 protein includes at least one of the following sequences:

- | | |
|----|--|
| | 1. the homologous sequence (H1) from <i>Lactuca sativa</i> as shown in SEQ ID NO: 24, |
| | 2. the homologous sequences (H2 and H3) from <i>Adonis palaestina</i> as shown in SEQ ID NO: 26 or 28, |
| | 3. the homologous sequence (H4) from <i>Arabidopsis thaliana</i> as shown in SEQ ID NO: 30 |
| | 4. the homologous sequences (H5 and H6) from <i>Citrus x paradisi</i> as shown in SEQ ID NO: 32 or 34 |
| 30 | 5. the homologous sequence (H7) from <i>Citrus sinensis</i> as shown in SEQ ID NO: 36 |
| | 6. the homologous sequence (H8) from <i>Spinacea oleracea</i> as shown in SEQ ID NO: 38 |
| | 7. the homologous sequence (H9) from <i>Solanum tuberosum</i> as shown in SEQ ID NO: 40 |
| | 8. the homologous sequences (H10 and H11) from <i>Daucus carota</i> as shown in SEQ ID NO: 42 or 44 |
| | 9. the homologous sequence (H12) from tomato as shown in SEQ ID NO: 46 |

40 Most preferred functional equivalents of the promoter described by SEQ ID NO: 1, 7 or 8 are those promoters which are located in a plant organism in the 5' direction in front of a genomic

sequence which codes for a nucleic acid sequence whose derived cDNA includes at least one of the following sequences:

1. the homologous sequence (H1) from *Lactuca sativa* as shown in SEQ ID NO: 23,
2. the homologous sequences (H2 and H3) from *Adonis palaestina* as shown in SEQ ID NO: 25 or 27,
3. the homologous sequence (H4) from *Arabidopsis thaliana* as shown in SEQ ID NO: 29
- 10 4. the homologous sequences (H5 and H6) from *Citrus x paradisi* as shown in SEQ ID NO: 31 or 33
7. the homologous sequence (H7) from *Citrus sinensis* as shown in SEQ ID NO: 35
5. the homologous sequence (H8) from *Spinacea oleracea* as shown in SEQ ID NO: 37
6. the homologous sequence (H9) from *Solanum tuberosum* as shown in SEQ ID NO: 39
8. the homologous sequences (H10 and H11) from *Daucus carota* as shown in SEQ ID NO: 41 or 43
- 20 9. the homologous sequence (H12) from tomato as shown in SEQ ID NO: 45.

Further examples of functionally equivalent promoter sequences employed in the transgenic expression cassettes or transgenic expression vectors of the invention can easily be found for example in various organisms whose genomic sequence is at least partly known, such as, for example, *Arabidopsis thaliana*, *Brassica napus*, *Nicotiana tabacum*, *Solanum tuberosum*, *Helianthus annuus*, *Linum sativum*, followed by homology comparisons in databases.

- 30 A further aspect of the invention relates to the use of at least one nucleic acid sequence or of a part thereof in methods for identifying and/or isolating promoters of genes which code for said nucleic acid sequence, where said nucleic acid sequence codes for an amino acid sequence which includes at least one sequence motif as shown in SEQ ID NO: 17, 18, 19, 20, 21 or 22 or a variation indicated for these sequence motifs. Said nucleic acid sequence preferably codes for an amino acid sequence including a sequence as shown in SEQ ID NO: 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46. Said nucleic acid sequence
- 40 particularly preferably includes a sequence as shown in SEQ ID NO: 23, 25, 27, 29, 29, 31, 33, 35, 37, 39, 41, 43 or 45. "Part" means in relation to the nucleic acid sequence preferably a sequence of at least 10 bases, preferably 15 bases, particularly preferably 20 bases, most preferably 30 bases.

- Further included according to the invention are methods for identifying and/or isolating promoters of genes which code for a promoter having specificity for the flower of plants, where at least one nucleic acid sequence or a part thereof is employed in the identification and/or isolation, where said nucleic acid sequence codes for an amino acid sequence which includes at least one sequence motif as shown in SEQ ID NO: 17, 18, 19, 20, 21 or 22 or a variation indicated for these sequence motifs. Said nucleic acid sequence preferably codes for an amino acid sequence including a sequence as shown in SEQ ID NO: 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46. Said nucleic acid sequence particularly preferably includes a sequence as shown in SEQ ID NO: 23, 25, 27, 29, 29, 31, 33, 35, 37, 39, 41, 43 or 45. "Part" means in relation to the nucleic acid sequence preferably a sequence of at least 10 bases, preferably 15 bases, particularly preferably 20 bases, most preferably 30 bases. In a preferred embodiment, the method of the invention is based on the polymerase chain reaction, where said nucleic acid sequence or a part thereof is employed as primer.
- 20 Various methods for identifying and isolating, starting from a nucleic acid sequence (e.g. a gene transcript such as, for example, a cDNA), the promoter of the corresponding gene are known to the skilled worker. In principle, all methods for amplifying flanking chromosomal sequences are available for example for this purpose. The two most commonly used methods are inverse PCR ("iPCR"; diagrammatically depicted in Fig. 13) and "thermal asymmetric interlaced PCR" ("TAIL PCR"). Also suitable in addition is the method of PCR walkings (Devic et al. (1997) Plant Physiol Biochem 35:331-339).
- 30 For the iPCR, genomic DNA of the organism from which the functionally equivalent promoter is to be isolated is completely digested with a given restriction enzyme, and then the individual fragments are religated, i.e. linked to themselves to give a circular molecule, in a diluted mixture. The large number of resulting circular DNA molecules also includes those comprising the known sequence (for example the sequence coding for the homologous protein). Starting from this, the circular molecule can be amplified by PCR using a primer pair where both primers are able to anneal to the known sequence segment. One possible
- 40 embodiment of the iPCR is reproduced in example 2.

The TAIL-PCR is based on the use of firstly a set of successively truncated highly specific primers which anneal to the known genomic sequence (for example the sequence coding for the

homologous protein), and secondly a set of shorter random primers with a lower melting temperature, so that a less sequence-specific annealing to genomic DNA flanking the known genomic sequence takes place. Annealing of the primers to the DNA to be amplified is possible with such a primer combination to make specific amplification of the desired target sequence possible. One possible embodiment of the TAIL-PCR is reproduced for example in example 2.

- 10 A further aspect of the invention relates to methods for preparing a transgenic expression cassette having specificity for the flowers of plants, including the following steps:

I. isolation of a promoter sequence, where at least one nucleic acid sequence or a part thereof is employed in the isolation, where said nucleic acid sequence codes for an amino acid sequence which includes at least one sequence motif as shown in SEQ ID NO: 17, 18, 19, 20, 21 or 22 or a variation indicated for these sequence motifs.

- 20 II. functional linkage of said promoter sequence to a further nucleic acid sequence, where said nucleic acid sequence is heterologous in relation to the promoter.

Said nucleic acid sequence preferably codes for an amino acid sequence including a sequence as shown in SEQ ID NO: 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46. Said nucleic acid sequence particularly preferably includes a sequence as shown in SEQ ID NO: 23, 25, 27, 29, 29, 31, 33, 35, 37, 39, 41, 43 or 45. "Part" means in relation to the nucleic acid sequence preferably a sequence of at least 10 bases, preferably 15 bases, particularly preferably 20 bases, most preferably 30 bases. In a preferred
30 embodiment, the method of the invention is based on the polymerase chain reaction, where said nucleic acid sequence or a part thereof is employed as primer. Methods known to the skilled worker, such as, for example, ligation etc., can be employed for the functional linkage (see below).

The level of expression of a functionally equivalent promoter can be both downwards and upwards compared with the promoter found in SEQ ID NO: 1, 7 or 8. Preference is given in this connection to the sequences whose level of expression, measured on the basis of the transcribed mRNA or the subsequently translated protein,
40 under conditions which are otherwise unchanged differs quantitatively by not more than 50%, preferably 25%, particularly preferably 10%, from a comparison value obtained with the

promoters described by SEQ ID NO: 1, 7 or 8. Particularly preferred sequences are those whose level of expression, measured on the basis of the transcribed mRNA or the subsequently translated protein, under conditions which are otherwise unchanged exceeds quantitatively by more than 50%, preferably 100%, particularly preferably 500%, very particularly preferably 1000%, a comparison value obtained with the promoter described by SEQ ID NO: 1, 7 or 8. The preferred comparison value is the level of expression of the mRNAs, naturally expressed from the

10 promoter, of an ϵ -cyclase or of the protein resulting therefrom. Also preferred as comparison value is the level of expression obtained with any defined nucleic acid sequence, preferably nucleic acid sequences which code for easily quantifiable proteins. Very particular preference is given in this connection to reporter proteins (Schenborn E & Groskreutz D (1999) Mol Biotechnol 13(1):29-44) such as the green fluorescent protein (GFP) (Chiu WL et al. (1996) Curr Biol 6:325-330; Leffell SM et al. (1997) Biotechniques. 23(5):912-8), chloramphenicol

20 acetyltransferase, a luciferase (Millar et al. (1992) Plant Mol Biol Rep 10:324-414) or β -glucuronidase, very particularly preferably β -glucuronidase (Jefferson et al. (1987) EMBO J 6:3901-3907).

Functional equivalents also include natural or artificial mutations of the promoter sequence described in SEQ ID NO: 1, 7 or 8. Mutations include substitutions, additions, deletions, inversions or insertions of one or more nucleotide residues. Thus, for example, the present invention also includes nucleotide sequences obtained by modification of the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8. The aim of such a modification may

30 be further localization of the sequence contained therein or, for example, also the insertion or deletion of restriction endonuclease cleavage sites, the deletion of excess DNA or the addition of further sequences, for example further regulatory sequences.

Where insertions, deletions or substitutions, such as, for example, transitions and transversions, are appropriate, it is possible to use techniques known per se, such as in vitro mutagenesis, primer repair, restriction or ligation. Transition means a base-pair exchange of a purine/pyrimidine pair into

40 another purine/pyrimidine pair (e.g. A-T for G-C). Transversion means a base-pair exchange of a purine/pyrimidine pair for a pyrimidine/purine pair (e.g. A-T for T-A). Deletion means removal of one or more base pairs. Insertion means introduction of one or more base pairs.

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Complementary ends of the fragments for ligation can be made available by manipulations such as, for example, restriction, chewing back or filling in of overhangs for blunt ends. Analogous results are also obtainable by using the polymerase chain reaction (PCR) using specific oligonucleotide primers.

10 Homology between two nucleic acids means the identity of the nucleic acid sequence over the complete sequence length in each case, which is calculated by comparison with the aid of the GAP program algorithm (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 12

Length Weight: 4

Average Match: 2.912

Average Mismatch:-2.003

20 For example, a sequence which has a homology of at least 50% based on nucleic acids with the sequence SEQ ID NO: 1 means a sequence which has a homology of at least 50% on comparison with the sequence SEQ ID NO: 1 by the above program algorithm with the above set of parameters.

Homology between two polypeptides means the identity of the amino acid sequence over the respective sequence length, which is calculated by comparison with the aid of the GAP program algorithm (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 8

Length Weight: 2

Average Match: 2.912

Average Mismatch:-2.003

30

For example, a sequence having a homology of at least 60% based on protein with the sequence SEQ ID NO: 10 means a sequence which has a homology of at least 60% on comparison with the sequence SEQ ID NO: 10 by the above program algorithm with the above set of parameters.

40 Functional equivalents also means DNA sequences which hybridize under standard conditions with the nucleic acid sequence coding for the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8, or with the nucleic acid sequences complementary thereto, and which have substantially the same promoter properties. The term standard hybridization conditions is to be understood broadly and

means both stringent and less stringent hybridization conditions. Such hybridization conditions are described inter alia in Sambrook J, Fritsch EF, Maniatis T et al., in Molecular Cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, 1989, pages 9.31-9.57 or in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

For example, the conditions during the washing step can be selected from the range of conditions limited by those of low stringency (with approximately 2X SSC at 50_C) and of high stringency (with approximately 0.2X SSC at 50_C, preferably at 65_C) (20X SSC: 0.3 M sodium citrate, 3 M NaCl, pH 7.0). In addition, the temperature during the washing step can be raised from low-stringency conditions at room temperature, approximately 22_C, to more stringent conditions at approximately 65_C. Both parameters, the salt concentration and the temperature, can be varied simultaneously, and it is also possible for one of the two parameters to be kept constant and only the other to be varied. It is also possible to employ denaturing agents such as, for example, formamide or SDS during the hybridization. Hybridization in the presence of 50% formamide is preferably carried out at 42_C. Some exemplary conditions for hybridization and washing steps are given below:

(1) Hybridization conditions with for example

- a) 4X SSC at 65_C, or
- b) 6X SSC, 0.5% SDS, 100 µg/ml denatured fragmented salmon sperm DNA at 65_C, or
- c) 4X SSC, 50% formamide, at 42_C, or
- d) 2X or 4X SSC at 50_C (low-stringency condition), or
- e) 2X or 4X SSC, 30 to 40% formamide at 42_C (low-stringency condition), or
- f) 6X SSC at 45_C, or,
- g) 0.05 M sodium phosphate buffer pH 7.0, 2 mM EDTA, 1% BSA and 7% SDS.

(2) Washing steps with for example

- a) 0.1X SSC at 65_C, or
- b) 0.1X SSC, 0.5% SDS at 68_C, or
- c) 0.1X SSC, 0.5% SDS, 50% formamide at 42_C, or
- d) 0.2X SSC, 0.1% SDS at 42_C, or
- e) 2X SSC at 65_C (low-stringency condition), or
- f) 40 mM sodium phosphate buffer pH 7.0, 1% SDS, 2 mM EDTA.

Methods for preparing functional equivalents of the invention preferably include the introduction of mutations into the ϵ -cyclase promoter as shown in SEQ ID NO: 1, 7 or 8. Mutagenesis may be random, in which case the mutagenized sequences are subsequently screened for their properties by a trial and error procedure. Particularly advantageous selection criteria include for example the level of the resulting expression of the introduced nucleic acid sequence in a flower tissue.

- 10 Methods for mutagenesis of nucleic acid sequences are known to the skilled worker and include by way of example the use of oligonucleotides with one or more mutations compared with the region to be mutated (e.g. in a site-specific mutagenesis). Primers with approximately 15 to approximately 75 nucleotides or more are typically employed, with preferably about 10 to about 25 or more nucleotide residues being located on both sides of the sequence to be modified. Details and procedure for said mutagenesis methods are familiar to the skilled worker (Kunkel et al. (1987) Methods Enzymol 154:367-382; Tomic et al. (1990) Nucl Acids Res 12:1656; Upender et al. (1995) Biotechniques 18(1):29-30; US 4,237,224). A mutagenesis can also be achieved by treating for example transgenic expression vectors comprising one of the nucleic acid sequences of the invention with mutagenizing agents such as hydroxylamine.

- An alternative possibility is to delete nonessential sequences of a promoter of the invention without significantly impairing the essential properties mentioned. Such deletion variants represent functionally equivalent fragments to the promoters described by SEQ ID NO: 1, 7 or 8 or to functional equivalents thereof.
- 30 Localization of the promoter sequence to particular essential regulatory regions can be carried out for example with the aid of search routine to search for promoter elements. Particular promoter elements are often present in increased numbers in the regions relevant for promoter activity. This analysis can be carried out for example with computer programs such as the PLACE program ("Plant Cis-acting Regulatory DNA Elements"; Higo K et al. (1999) Nucl Acids Res 27(1): 297-300), the BIOBASE database "Transfac" (Biologische Datenbanken GmbH, Braunschweig; Wingender E et al. (2001) Nucleic Acids Res 29(1):281-3) or the PlantCARE database (Lescot M et al. (2002) Nucleic Acids Res 30(1):325-7).
- 40

The functionally equivalent fragments of one of the promoters of the invention - for example of the ϵ -cyclase promoters described by SEQ ID NO: 1, 7 or 8 - preferably include at least 200 base

pairs, very particularly preferably at least 500 base pairs, most preferably at least 1000 base pairs of the 3' end of the respective promoter of the invention - for example the promoters described by SEQ ID NO: 1, 7 or 8 - the length being calculated from the translation start ("ATG" codon) upstream in the 5' direction.

10 Further functionally equivalent fragments may be generated for example by deleting any 5'-untranslated regions still present. For this purpose, the start of transcription of the corresponding genes can be determined by methods familiar to the skilled worker (such as, for example, 5'-RACE), and the 5'-untranslated regions can be deleted by PCR-mediated methods or endonuclease digestion. Thus, for example, the 5'-untranslated regions included in the promoters shown in SEQ ID NO: 7 or 8 can be deleted without the promoter losing its essential functionality. Corresponding deletion variants are expressly included as functional equivalents.

20 In transgenic expression cassettes of the invention, at least one of the promoters of the invention (e.g. described by SEQ ID NO: 1, 7 or 8) is functionally linked to at least one nucleic acid sequence to be expressed transgenically.

30 A functional linkage means, for example, the sequential arrangement of one of the promoters of the invention (e.g. described by SEQ ID NO: 1, 7 or 8) with a nucleic acid sequence to be expressed transgenically and, where appropriate, further genetic control sequences such as, for example, a terminator or a polyadenylation sequence in such a way that the promoter is able to fulfill its function in the transgenic expression of the nucleic acid sequence under suitable conditions, and expression of the nucleic acid sequence (i.e. transcription and, where appropriate, translation) takes place. "Suitable conditions" means in this connection preferably the presence of the expression cassette in a plant cell, preferably a plant cell included in a flower of the plant.

40 Arrangements in which the nucleic acid sequence to be expressed transgenically is positioned behind one of the promoters of the invention (e.g. described by SEQ ID NO: 1, 7 or 8), so that the two sequences are covalently connected together, are preferred. In this connection, the distance between the promoter sequence and the nucleic acid sequence to be expressed transgenically is preferably fewer than 200 base pairs, particularly preferably

less than 100 base pairs, very particularly preferably less than 50 base pairs.

Production of a functional linkage and production of a transgenic expression construct can be achieved by means of conventional recombination and cloning techniques as described for example in Maniatis T, Fritsch EF and Sambrook J (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Silhavy TJ, Berman ML and Enquist LW (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY) and in Ausubel FM et al. (1987) Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience. However, further sequences which have for example the function of a linker with particular restriction enzyme cleavage sites or of a signal peptide may also be positioned between the two sequences. Insertion of sequences may also lead to expression of fusion proteins. It is possible and preferred for the transgenic expression construct, consisting of a linkage of promoter and nucleic acid sequence to be expressed, to be integrated into a vector and be inserted into a plant genome for example by transformation.

However, an expression cassette also means constructions in which one of the promoters of the invention (e.g. described by SEQ ID NO: 1, 7 or 8) is, without necessarily having been functionally linked beforehand to a nucleic acid sequence to be expressed, introduced into a host genome, for example by targeted homologous recombination or random insertion, there undertakes regulatory control over endogenous nucleic acid sequences then functionally linked thereto, and controls the transgenic expression thereof. Insertion of the promoter - for example by a homologous recombination - in front of a nucleic acid coding for a particular polypeptide results in an expression cassette of the invention which controls the targeted expression of the particular polypeptide in the flower of plants. It is also possible for example for the natural promoter of an endogenous gene to be replaced by one of the promoters of the invention (e.g. described by SEQ ID NO: 1, 7 or 8), and for the expression behavior of the endogenous gene to be modified.

A further possibility is also for the promoter to be inserted in such a way that antisense RNA or a double-stranded RNA (e.g. in the form of an inverted repeat) is expressed to give the nucleic acid coding for a particular polypeptide. In this way, expression of the particular polypeptide in the flower of plants is selectively downregulated or switched off.

It is also possible analogously for a nucleic acid sequence which is to be expressed transgenically to be placed - for example by homologous recombination - behind the sequence which codes for one of the promoters of the invention (e.g. described by SEQ ID NO: 1, 7 or 8), and which is located in its natural chromosomal context, so as to result in an expression cassette of the invention which controls the expression of the nucleic acid sequence to be expressed transgenically in the flower of plants.

10 The transgenic expression cassettes of the invention may include further genetic control sequences. The term genetic control sequences is to be understood broadly and means all sequences having an influence on the coming into existence or the function of a transgenic expression cassette of the invention. Genetic control sequences modify for example the transcription and translation in prokaryotic or eukaryotic organisms. The transgenic expression cassettes of the invention preferably include as additional genetic control sequence a terminator sequence 3'-downstream from the particular nucleic acid sequence to be expressed transgenically, and where appropriate further
20 customary regulatory elements, in each case functionally linked to the nucleic acid sequence to be expressed transgenically.

Genetic control sequences also include further promoters, promoter elements or minimal promoters able to modify the expression-controlling properties. It is thus possible for example through genetic control sequences for tissue-specific expression to take place additionally in dependence on particular stress factors. Corresponding elements are described for example for water stress, abscisic acid (Lam E and Chua NH, J Biol Chem 1991; 266(26):17131-17135) and heat stress (Schoffl F et al.
30 (1989) Mol Gen Genetics 217(2-3):246-53).

A further possibility is for further promoters which make transgenic expression possible in further plant tissues or in other organisms such as, for example, *E.coli* bacteria to be functionally linked to the nucleic acid sequence to be expressed. Suitable promoters are in principle all promoters functional in plants. Promoters functional in plants means in principle every promoter able to control the expression of genes, in particular foreign genes, in plants or plant parts, cells, tissues, cultures. It is moreover possible for expression to be for
40 example constitutive, inducible or development-dependent. Preference is given to constitutive promoters, tissue-specific promoters, development-dependent promoters, chemically inducible,

stress-inducible or pathogen-inducible promoters. Corresponding promoters are generally known to the skilled worker.

Further advantageous control sequences are to be found for example in the promoters of gram-positive bacteria such as amy and SPO2 or in the yeast or fungal promoters ADC1, MF α , AC, P-60, CYC1, GAPDH, TEF, rp28, ADH.

10 It is possible in principle for all natural promoters with their regulatory sequences like those mentioned above to be used for the method of the invention. It is additionally also possible for synthetic promoters to be used advantageously.

20 Genetic control sequences further include also the 5'-untranslated regions, introns or noncoding 3' region of genes such as, for example, the actin-1 intron, or the Adh1-S introns 1, 2 and 6 (generally: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)), preferably the genes with the gene locus At2g46720, At3g01980 and At1g63140 from Arabidopsis thaliana. It is possible to show that such regions may have a significant function in regulating gene expression. Thus, it has been shown that 5'-untranslated sequences are able to enhance the transient expression of heterologous genes. Examples of translation enhancers which may be mentioned are the 5' leader sequence from tobacco mosaic virus (Gallie et al. (1987) Nucl Acids Res 15:8693-8711) and the like. They may in addition promote tissue specificity (Rouster J et al. (1998) Plant J 15:435-440). The nucleic acid sequences indicated in SEQ ID NO: 2, 7 or 8 in each case represent the promoter region and the 5'-untranslated regions up to the ATG start codon of the respective genes.

30 The transgenic expression construct may advantageously comprise one or more so-called enhancer sequences functionally linked to the promoter, which make increased transgenic expression of the nucleic acid sequence possible. Additional advantageous sequences can also be inserted at the 3' end of the nucleic acid sequences to be expressed transgenically, such as further regulatory elements or terminators. The nucleic acid sequences to be expressed transgenically may be present in one or more copies in the gene construct.

40 Polyadenylation signals suitable as control sequences are plant polyadenylation signals, preferably those which are essentially T-DNA polyadenylation signals from Agrobacterium tumefaciens. Examples of particularly suitable terminator sequences are the

OCS (octopine synthase) terminator and the NOS (nopaline synthase) terminator.

Control sequences additionally mean those which make homologous recombination or insertion into the genome of a host organism possible or allow deletion from the genome. In homologous recombination for example the coding sequence of a particular endogenous gene can be specifically replaced by a sequence coding for a dsRNA. Methods such as cre/lox technology permit tissue-specific, and in some circumstances inducible, deletion of the transgenic expression constant from the genome of the host organism (Sauer B (1998) Methods 14(4):381-92). In this case, particular flanking sequences are attached to the target gene (lox sequences) and make later deletion by means of cre recombinase possible.

A transgenic expression cassette and/or the transgenic expression vectors derived therefrom may comprise further functional elements. The term functional element is to be understood broadly and means all elements which have an influence on the production, replication or function of the transgenic expression constructs of the invention, of the transgenic expression vectors or of the transgenic organisms. Non-restrictive examples which may be mentioned are:

- a) Selection markers which confer resistance to biocides such as metabolism inhibitors (e.g. 2-deoxyglucose 6-phosphate; WO 98/45456), antibiotics (e.g. kanamycin, G 418, bleomycin, hygromycin) or - preferably - herbicides (e.g. phosphinothricin). Examples of selection markers which may be mentioned are: phosphinothricin acetyltransferases (bar and pat gene), which inactivate glutamine synthase inhibitors, 5-enolpyruvylshikimate-3-phosphate synthases (EPSP synthase genes) which confer resistance to glyphosate (N-(phosphonomethyl)glycine), glyphosate-degrading enzymes (gox gene product; glyphosate oxidoreductase), dehalogenases which for example inactivate dalapon (deh gene product), sulfonylurea- and imidazolinone-inactivating acetolactate synthases, and nitrilases which for example degrade bromoxynil (bxn gene product), the aasa gene product which confers resistance to the antibiotic spectinomycin, streptomycin phosphotransferases (SPT) which ensure resistance to streptomycin, neomycin phosphotransferases (NPTII) which confer resistance to kanamycin or geneticin, the hygromycin phosphotransferases (HPT) which mediate resistance to hygromycin, the acetolactate synthases (ALS)

which confer resistance to sulfonylurea herbicides (e.g. mutated ALS variants with, for example, the S4 and/or Hra mutation).

- 10 b) Reporter genes which code for easily quantifiable proteins and ensure via an intrinsic color or enzymic activity an assessment of the transformation efficiency or of the location or timing of expression. Very particular preference is given in this connection to reporter proteins (Schenborn E, Groskreutz D. Mol Biotechnol. 1999; 13(1):29-44) such as the green fluorescent protein (GFP) (Sheen et al. (1995) Plant Journal 8(5):777-784), the chloramphenicol acetyltransferase, a luciferase (Ow et al. (1986) Science 234:856-859), the aequorin gene (Prasher et al. (1985) Biochem Biophys Res Commun 126(3):1259-1268), the β -galactosidase, with very particular preference for β -glucuronidase (Jefferson et al. (1987) EMBO J 6:3901-3907).
- 20 c) Origins of replication which ensure replication of the transgenic expression constructs or transgenic expression vectors of the invention in, for example, E. coli. Examples which may be mentioned are ORI (origin of DNA replication), the pBR322 ori or the P15A ori (Sambrook et al.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).
- d) Elements which are necessary for agrobacterium-mediated plant transformation, such as, for example, the right or left border of the T-DNA or the vir region.

30 "Introduction" includes for the purposes of the invention all methods suitable for introducing a nucleic acid sequence (for example an expression cassette of the invention) directly or indirectly into an organism (e.g. a plant) or a cell, compartment, tissue, organ or propagation material (e.g. seeds or fruits) thereof, or for generating such therein. Direct and indirect methods are included. The introduction can lead to a temporary (transient) presence of said nucleic acid sequence or else to a permanent (stable) presence. Introduction includes for example methods such as transfection, transduction or transformation. The organisms used in the methods are grown or cultured, depending on the host organism, in the manner known to

40 the skilled worker.

Introduction of a transgenic expression cassette of the invention into an organism or cells, tissues, organs, parts or seeds

thereof (preferably into plants or plant cells, tissues, organs, parts or seeds) can advantageously be achieved by use of vectors comprising the transgenic expression cassettes. Vectors may be for example plasmids, cosmids, phages, viruses or else agrobacteria. The transgenic expression cassettes can be inserted into the vector (preferably a plasmid vector) via a suitable restriction cleavage site. The resulting vector can be firstly introduced and amplified in *E. coli*. Correctly transformed *E. coli* are selected and cultured, and the recombinant vector is isolated by methods familiar to the skilled worker. Restriction analysis and sequencing can be used to check the cloning step. Preferred vectors are those making stable integration of the expression cassette into the host genome possible.

Production of a transformed organism (or of a transformed cell or tissue) requires introduction of the appropriate DNA (e.g. the expression vector) or RNA into the appropriate host cell. A large number of methods is available for this process, which is referred to as transformation (or transduction or transfection) (Keown et al. (1990) *Methods in Enzymology* 185:527-537). Thus, the DNA or RNA can for example be introduced directly by microinjection or by bombardment with DNA-coated microparticles. The cell can also be permeabilized chemically, for example with polyethylene glycol, so that the DNA is able to enter the cell by diffusion. The DNA introduction can also take place by protoplast fusion with other DNA-containing units such as minicells, cells, lysosomes or liposomes. Electroporation is another suitable method for introducing DNA, in which the cells are reversibly permeabilized by an electrical impulse. Corresponding methods are described (for example in Bilang et al. (1991) *Gene* 100:247-250; Scheid et al. (1991) *Mol Gen Genet* 228:104-112; Guerche et al. (1987) *Plant Science* 52:111-116; Neuhaase et al. (1987) *Theor Appl Genet* 75:30-36; Klein et al. (1987) *Nature* 327:70-73; Howell et al. (1980) *Science* 208:1265; Horsch et al. (1985) *Science* 227:1229-1231; DeBlock et al. (1989) *Plant Physiology* 91:694-701; *Methods for Plant Molecular Biology* (Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and *Methods in Plant Molecular Biology* (Schuler and Zielinski, eds.) Academic Press Inc. (1989)).

Vectors preferred for expression in *E. coli* are pQE70, pQE60 and pQE-9 (QIAGEN, Inc.); pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene Cloning Systems, Inc.); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia Biotech, Inc.).

Preferred vectors for expression in mammalian cells include pWLNE0, pSV2CAT, pOG44, pXT1 and pSG (Stratagene Inc.); pSVK3, pBPV, pMSG and pSVL (Pharmacia Biotech, Inc.). Inducible vectors which may be mentioned are pTet-tTak, pTet-Splice, pcDNA4/TO, pcDNA4/TO /LacZ, pcDNA6/TR, pcDNA4/TO/Myc-His/LacZ, pcDNA4/TO/Myc-His A, pcDNA4/TO/Myc-His B, pcDNA4/TO/Myc-His C, pVgRXR (Invitrogen, Inc.) or the pMAM series (Clontech, Inc.; GenBank Accession No: U02443). These themselves provide the inducible regulatory control element for example for a chemically
 10 inducible expression.

Vectors for expression in yeast include for example pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, PHIL-D2, PHIL-S1, pPIC3SK, pPIC9K, and PA0815 (Invitrogen, Inc.).

Cloning vectors and techniques for genetic manipulation of ciliates and algae are known to the skilled worker (WO 98/01572; Falciatore et al. (1999) Marine Biotechnology 1(3):239-251; Dunahay et al. (1995) J Phycol 31:10004-1012).

The methods to be used in principle for the transformation of animal cells or of yeast cells are similar to those for "direct"
 20 transformation of plant cells. Methods such as calcium phosphate or liposome-mediated transformation or else electroporation are preferred in particular.

Various methods and vectors for inserting genes into the genome of plants and for regenerating plants from plant tissues or plant cells are known (Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, pp. 71-119 (1993); White FF (1993) Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, editors: Kung and Wu R, Academic Press, 15-38; Jenes B et al. (1993)
 30 Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, editors: Kung and R. Wu, Academic Press, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225; Halford NG, Shewry PR (2000) Br Med Bull 56(1):62-73). Those mentioned above are included, for example. In the case of plants, the described methods for the transformation and regeneration of plants from plant tissues or plant cells are used for transient or stable transformation. Suitable methods are, in particular, protoplast transformation by polyethylene glycol-induced DNA uptake, calcium phosphate-mediated
 40 transformation, DEAE-dextran-mediated transformation, liposome-mediated transformation (Freeman et al. (1984) Plant Cell Physiol. 29:1353ff; US 4,536,475), biolistic methods with the

gene gun ("particle bombardment" method; US 5,100,792; EP-A 0 444 882; EP-A 0 434 616; Fromm ME et al. (1990) Bio/Technology 8(9):833-9; Gordon-Kamm et al. (1990) Plant Cell 2:603), electroporation, incubation of dry embryos in DNA-containing solution, electroporation (EP-A 290 395, WO 87/06614), microinjection (WO 92/09696, WO 94/00583, EP-A 0 331 083, EP-A 0 175 966) or other methods of direct DNA introduction (DE 4 005 152, WO 90/12096, US 4,684,611). Physical methods of DNA introduction into plant cells are surveyed in Oard (1991) Biotech
 10 Adv 9:1-11.

In the case of these "direct" transformation methods, no particular requirements need be met by the plasmid used. Simple plasmids such as those of the pUC series, pBR322, M13mp series, pACYC184 etc. can be used. If complete plants are to be regenerated from the transformed cells, it is necessary for an additional selectable marker gene to be present on the plasmid.

Besides these "direct" transformation techniques, it is also possible to carry out a transformation by bacterial infection using agrobacterium (e.g. EP 0 116 718), viral infection using
 20 viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or using pollen (EP 0 270 356; WO 85/01856; US 4,684,611).

The transformation is preferably effected using agrobacteria which comprise disarmed Ti plasmid vectors, utilizing their natural ability to transfer genes to plants (EP-A 0 270 355; EP-A 0 116 718).

Agrobacterium transformation is widely used for the transformation of dicotyledons, but is also increasingly being applied to monocotyledons (Toriyama et al. (1988) Bio/Technology
 30 6: 1072-1074; Zhang et al. (1988) Plant Cell Rep 7:379-384; Zhang et al. (1988) Theor Appl Genet 76:835-840; Shimamoto et al. (1989) Nature 338:274-276; Datta et al. (1990) Bio/Technology 8: 736-740; Christou et al. (1991) Bio/Technology 9:957-962; Peng et al. (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao et al. (1992) Plant Cell Rep 11:585-591; Li et al. (1993) Plant Cell Rep 12:250-255; Rathore et al. (1993) Plant Mol Biol 21:871-884; Fromm et al. (1990) Bio/Technology 8:833-839; Gordon-Kamm et al. (1990) Plant Cell 2:603-618; D'Halluin et al. (1992) Plant Cell 4:1495-1505; Walters et al.
 40 (1992) Plant Mol Biol 18:189-200; Koziel et al. (1993) Biotechnology 11:194-200; Vasil IK (1994) Plant Mol Biol 25:925-937; Weeks et al. (1993) Plant Physiol 102:1077-1084; Somers et

al. (1992) Bio/Technology 10:1589-1594; WO 92/14828; Hiei et al. (1994) Plant J 6:271-282).

10 The strains mostly used for agrobacterium transformation, *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* comprise a plasmid (Ti or Ri plasmid) which is transferred to the plant after agrobacterium infection. Part of this plasmid, called T-DNA (transferred DNA), is integrated into the genome of the plant cell. Alternatively, binary vectors (mini-Ti plasmids) can also be transferred into plants and integrated in the genome thereof by agrobacterium.

20 The use of *Agrobacterium tumefaciens* for the transformation of plants using tissue culture explants is described (inter alia Horsch RB et al. (1985) Science 225:1229ff.; Fraley et al. (1983) Proc Natl Acad Sci USA 80: 4803-4807; Bevens et al. (1983) Nature 304:184-187). Many *Agrobacterium tumefaciens* strains are able to transfer genetic material - for example the expression cassettes of the invention - such as, for example, the strains EHA101[pEHA101], EHA105[pEHA105], LBA4404[pAL4404], C58C1[pMP90] and C58C1[pGV2260] (Hood et al. (1993) Transgenic Res 2:208-218; Hoekema et al. (1983) Nature 303:179-181; Koncz and Schell (1986) Gen Genet 204:383-396; Deblaere et al. (1985) Nucl Acids Res 13: 4777-4788).

30 On use of agrobacteria, the expression cassette must be integrated into specific plasmids either into a shuttle or intermediate vector or into a binary vector. Binary vectors able to replicate both in *E. coli* and in agrobacterium are preferably used. They normally comprise a selection marker gene and a linker or polylinker, flanked by the right and left T-DNA border sequence. They can be transformed directly into agrobacterium (Holsters et al. (1978) Mol Gen Genet 163:181-187). The agrobacterium acting as host organism in this case should already comprise a plasmid having the vir region. This is necessary for transfer of the T-DNA into the plant cell. An agrobacterium transformed in this way can be used to transform plant cells. The use of T-DNA for transforming plant cells has been intensively investigated and described (EP-A 0 120 516; Hoekema, In: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasterdam, Chapter V; An et al. (1985) EMBO J 4:277-287). Various binary vectors are known, and some of them are
40 commercially available, such as, for example, pBI101.2 or pBIN19 (Clontech Laboratories, Inc. USA; Bevan et al. (1984) Nucl Acids Res 12:8711), pBinAR, pPZP200 or pPTV.

Agrobacteria transformed with such a vector can then be used in a known manner for transforming plants, especially crop plants such as, for example, oilseed rape, by for example bathing wounded leaves or pieces of leaf in a solution of agrobacteria and then cultivating in suitable media. Transformation of plants by agrobacteria is described (White FF (1993) Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, edited by SD Kung and R Wu, Academic Press, pp. 15-38; Jenes B et al. (1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225). Transgenic plants which have integrated the expression systems of the invention described above can be regenerated in a known manner from the transformed cells of the wounded leaves or pieces of leaf.

Stably transformed cells (i.e. those which have integrated the introduced DNA into the DNA of the host cell) can be selected from untransformed ones if a selectable marker is a constituent of the introduced DNA. Any gene able to confer a resistance to a biocide (e.g. an antibiotic or herbicide, see above) can act as marker, for example. Transformed cells which express such a marker gene are able to survive in the presence of concentrations of a corresponding biocide which kill an untransformed wild type. The selection marker permits the selection of transformed cells from untransformed ones (McCormick et al. (1986) Plant Cell Reports 5:81-84). The resulting plants can be grown and crossed in the usual way. Two or more generations should be cultivated in order to ensure that the genomic integration is stable and heritable.

As soon as a transformed plant cell has been produced, it is possible to obtain a complete plant by using methods known to the skilled worker. These entail, for example, starting from callus cultures, single cells (e.g. protoplasts) or leaf disks (Vasil et al. (1984) Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications, Academic Press; Weissbach and Weissbach (1989) Methods for Plant Molecular Biology, Academic Press). The formation of shoot and root from these still undifferentiated callus cell masses can be induced in a known manner. The resulting shoots can be planted out and grown. Corresponding methods are described (Fennell et al. (1992) Plant Cell Rep. 11: 567-570; Stoeger et al (1995) Plant Cell Rep. 14:273-278; Jahne et al. (1994) Theor Appl Genet 89:525-533).

The effectiveness of expression of the transgenically expressed nucleic acids can be estimated for example in vitro by shoot-meristem propagation using one of the selection methods described above. In addition, a change in the type and level of expression of a target gene, and the effect on the phenotype of the plant can be tested on test plants in glasshouse tests.

10 A further aspect of the invention relates to transgenic organisms transformed with at least one expression cassette of the invention or one vector of the invention, and cells, cell cultures, tissues, parts - such as, for example, in the case of plant organisms leaves, roots etc. - or propagation material derived from such organisms.

By organism, starting or host organisms are meant prokaryotic or eukaryotic organisms such as, for example, microorganisms or plant organisms. Preferred microorganisms are bacteria, yeasts, algae or fungi.

20 Preferred bacteria are bacteria of the genus *Escherichia*, *Erwinia*, *Agrobacterium*, *Flavobacterium*, *Alcaligenes*, *Pseudomonas*, *Bacillus* or cyanobacteria, for example of the genus *Synechocystis* and further bacterial genera described in Brock Biology of Microorganisms Eighth Edition on pages A-8, A-9, A10 and A11.

30 Microorganisms which are particularly preferred are those able to infect plants and thus transfer the constructs of the invention. Preferred microorganisms are those of the genus *Agrobacterium* and especially of the species *Agrobacterium tumefaciens*. Particularly preferred microorganisms are those able to produce toxins (e.g. botulinum toxin), pigments (e.g. carotenoids or flavonoids), antibiotics (e.g. penicillin), phenylpropanoids (e.g. tocopherol), polyunsaturated fatty acids (e.g. arachidonic acid) or vitamins (e.g. vitamin B12).

Preferred yeasts are *Candida*, *Saccharomyces*, *Hansenula* or *Pichia*.

Preferred fungi are *Aspergillus*, *Trichoderma*, *Ashbya*, *Neurospora*, *Fusarium*, *Beauveria* or further fungi described in Indian Chem Engr. Section B. Vol 37, No. 1,2 (1995) on page 15, table 6.

Host or starting organisms preferred as transgenic organisms are in particular plant organisms.

"Plant organism of cells derived therefrom" means in general every cell, tissue, part or propagation material (such as seeds or fruits) of an organism capable of photosynthesis. Included for the purposes of the invention are all genera and species of higher and lower plants of the plant kingdom. Annual, perennial, monocotyledonous and dicotyledonous plants are preferred.

10 "Plant" means for the purposes of the invention all genera and species of higher and lower plants of the plant kingdom. The term includes the mature plants, seeds, shoots and seedlings, and parts derived therefrom, propagation material (for example tubers, seeds or fruits), plant organs, tissues, protoplasts, callus and other cultures, for example cell or callus cultures, and all other types of groupings of plant cells to functional or structural units. Mature plants means plants at any stage of development beyond seedling. Seedling means a young, immature plant at an early stage of development.

20 Plant organisms for the purposes of the invention are additionally further photosynthetically active organisms such as, for example, algae, cyanobacteria and mosses. Preferred algae are green algae, such as, for example, algae of the genus *Haematococcus*, *Phaedactylum tricornatum*, *Volvox* or *Dunaliella*. *Synechocystis*, *Chlamydomonas* and *Scenedesmus* are particularly preferred.

30 Particularly preferred for the purposes of the method of the invention are plant organisms selected from the group of flowering plants (Phylum Anthophyta "angiosperms"). All annual and perennial, monocotyledonous and dicotyledonous plants are included. The plant is preferably selected from the following plant families: *Amaranthaceae*, *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Compositae*, *Cruciferae*, *Cucurbitaceae*, *Labiatae*, *Leguminosae*, *Papilionoideae*, *Liliaceae*, *Linaceae*, *Malvaceae*, *Rosaceae*, *Rubiaceae*, *Saxifragaceae*, *Scrophulariaceae*, *Solanaceae*, *Sterculiaceae*, *Tetragoniaceae*, *Theaceae* and *Umbelliferae*.

The invention is very particularly preferably applied to dicotyledonous plant organisms. Preferred dicotyledonous plants are in particular selected from the dicotyledonous crop plants such as, for example the following

1) Category: *Dicotyledonae* (dicotyledons). Preferred families:

40 - *Aceraceae* (maples)

- Cactaceae (cacti)
- Rosaceae (roses, apples, almonds, strawberries)
- Salicaceae (willows)
- Asteraceae (compositae) especially the genus *Lactuca*, very especially the species *sativa* (lettuce), and sunflower, dandelion, *Tagetes* or *Calendula* and many others,
- 10 - Cruciferae (Brassicaceae), especially the genus *Brassica*, very especially the species *napus* (oilseed rape), *campestris* (beet), oleracea (e.g. cabbage, cauliflower or broccoli and other brassica species); and of the genus *Arabidopsis*, very especially the species *thaliana*, and cress, radish, canola and many others,
- Cucurbitaceae such as melon, pumpkin, cucumber or zucchini and many others,
- Leguminosae (Fabaceae) especially the genus *Glycine*, very especially the species *max* (soybean), soya and alfalfa, pea,
- 20 beans, lupin or peanut and many others,
- Malvaceae, especially mallow, cotton, edible marshmallow, hibiscus and many others,
- Rubiaceae, preferably of the subclass Lamiidae such as, for example, *Coffea arabica* or *Coffea liberica* (coffee bush) and many others,
- Solanaceae, especially the genus *Lycopersicon*, very especially the species *esculentum* (tomato) and the genus *Solanum*, very especially the species *tuberosum* (potato) and *melongena* (eggplant) and the genus *Capsicum*, very especially the species
- 30 *annuum* (paprika), and tobacco, petunia and many others,
- Sterculiaceae, preferably of the subclass Dilleniidae such as, for example, *Theobroma cacao* (cocoa plant) and many others,
- Theaceae, preferably of the subclass Dilleniidae such as, for example, *Camellia sinensis* or *Thea sinensis* (tea bush) and many others,

- Umbelliferae (Apiaceae), especially the genus *Daucus* (very especially the species *carota* (carrot)), *Apium* (very especially the species *graveolens dulce* (celeriac)), and parsley and many others;

and flax, hemp, spinach, carrot, sugarbeet and the various tree, nut and vine species, especially bannana and kiwi fruit.

- However, in addition, monocotyledonous plants are also suitable. These are preferably selected from the monocotylendonous crop
- 10 plants such as, for example the families

- Arecaceae (palms)
- Bromeliaceae (pineapple, spanish moss)
- Cyperaceae (sedges)
- Liliaceae (lilies, tulips, hyacinths, onions, garlic)
- Orchidaceae (orchids)
- Poaceae (grasses, bamboos, corn, sugarcane, wheat)
- Iridaceae (buckwheat, gladioli, crocuses)

- Very particular preference is given to Gramineae such as rice,
- 20 corn, wheat or other cereal species such as barley, millet, rye, triticale or oats, and the sugarcane, and all species of grasses.

Within the framework of the expression cassette of the invention, expression of a particular nucleic acid may, through a promoter having specificity for the flower of plants, lead to the formation of sense RNA, antisense RNA or double-stranded RNA in the form of an inverted repeat (dsRNAi). The sense RNA can subsequently be translated into particular polypeptides. It is possible with the antisense RNA and dsRNAi to down regulate the expression of particular genes.

- 30 The method of gene regulation by means of double-stranded RNA ("double-stranded RNA interference"; dsRNAi) has been described in animal and plant organisms many times (e.g. Matzke MA et al. (2000) *Plant Mol Biol* 43:401-415; Fire A et al (1998) *Nature* 391:806-811; WO 99/32619; WO 99/53050; WO 00/68374; WO 00/44914; WO 00/44895; WO 00/49035; WO 00/63364). Express reference is made to the processes and methods described in the citations indicated.

- The specificity of the expression constructs and vectors of the invention for flowers of plants is particularly advantageous. The
- 40 flower has the function in attracting beneficial insects through incorporation of pigments or synthesis of volatile chemicals.

The natural defense mechanisms of the plant, for example against pathogens, are often inadequate. Introduction of foreign genes from plants, animals or microbial sources may enhance the defenses. Examples are protection against insect damage to tobacco through expression of the *Bacillus thuringiensis* endotoxin (Vaeck et al. (1987) *Nature* 328:33-37) or protection of tobacco from fungal attack through expression of a chitinase from beans (Broglie et al. (1991) *Science* 254:1194-1197).

- 10 Cold spells during the flowering period lead to considerable crop losses every year. Targeted expression of protective proteins specifically in the flowering period may provide protection.

For such genetic engineering approaches to be highly efficient it is advantageous for there to be concentrated expression of the appropriate nucleic acid sequence to be expressed transgenically in particular in the petals of the flower. Constitutive expression in the whole plant may make the effect problematic, for example through dilution, or impair the growth of the plant or the quality of the plant product. In addition, there may through constitutive expression be increased switching-off of the transgene ("gene silencing").

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Promoters having specificity for the flower are advantageous in this connection. The skilled worker is aware of a large number of proteins whose recombinant expression in the flower is advantageous. The skilled worker is also aware of a large number of genes through which advantageous effects can likewise be achieved through repression or switching-off thereof by means of expression of a corresponding antisense RNA. Non-restrictive examples of advantageous effects which may be mentioned are: achieving resistance to abiotic stress factors (heat, cold, aridity, increased moisture, environmental toxins, UV radiation) and biotic stress factors (pathogens, viruses, insects and diseases), improving the properties of human and animal foods, improving the growth rate or the yield, achieving a longer or earlier flowering period, altering or enhancing the scent or the coloring of the flowers. Non-restrictive examples of the nucleic acid sequences or polypeptides which can be employed in these applications and which may be mentioned are:

30

1. Improved UV protection of the flowers of plants through alteration of the pigmentation through expression of particular polypeptides such as enzymes or regulators of flavonoid biosynthesis (e.g. chalcone synthases, phenylalanine ammonia-lyases), of DNA repair (e.g.
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photolyases; Sakamoto A et al. (1998) DNA Seq 9(5-6):335-40), of isoprenoid biosynthesis (e.g. deoxyxylulose-5-phosphate synthases), of IPP synthesis or of carotenoid biosynthesis (e.g. phytoene synthases, phytoene desaturases, lycopene cyclases, hydroxylases or ketolases). Preference is given to nucleic acids which code for the Arabidopsis thaliana chalcone synthase (GenBank Acc. No.: M20308), the Arabidopsis thaliana 6-4 photolyase (GenBank Acc. No.: BAB00748) or the Arabidopsis thaliana blue light photoreceptor/photolyase homolog (PHH1) (GenBank Acc. No.: U62549) or functional equivalents thereof.

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2. Improved protection of the flower of plants from abiotic stress factors such as aridity, heat or cold, for example through overexpression of the antifreeze polypeptides (e.g. from Myoxocephalus scorpius; WO 00/00512), of the Arabidopsis thaliana transcription activator CBF1, glutamate dehydrogenases (WO 97/12983, WO 98/11240), a late embryogenesis gene (LEA), for example from barley (WO 97/13843), calcium-dependent protein kinase genes (WO 98/26045), calcineurins (WO 99/05902), farnesyl transferases (WO 99/06580; Pei ZM et al. (1998) Science 282:287-290), ferritin (Deak M et al. (1999) Nature Biotechnology 17:192-196), oxalate oxidase (WO 99/04013; Dunwell JM (1998) Biotechnology and Genetic Engineering Reviews 15:1-32), DREB1A factor (dehydration response element B 1A; Kasuga M et al. (1999) Nature Biotechnology 17:276-286), genes of mannitol or trehalose synthesis (e.g. trehalose-phosphate synthases; trehalose-phosphate phosphatases, WO 97/42326); or through inhibition of genes such as of trehalase (WO 97/50561). Particular preference is given to nucleic acids which code for the Arabidopsis thaliana transcriptional activator CBF1 (Gen-Bank Acc. No.: U77378) or the antifreeze protein from Myoxocephalus octodecemspinosus (GenBank Acc. No.: AF306348) or functional equivalents thereof.

3. Achieving resistance for example to fungi, insects, nematodes and diseases through targeted secretion or accumulation of certain metabolites or proteins in the flower. Examples which may be mentioned are glucosinolates (nematode defense), chitinases or glucanases and other enzymes which destroy the cell wall of parasites, ribosome-inactivating proteins (RIPs) and other proteins of the plant resistance and stress response, like those induced on injury or microbial attack of plants or chemically by, for example, salicylic acid,

- jasmonic acid or ethylene, lysozymes from non-plant sources such as, for example, T4 lysozyme or lysozyme from various mammals, insecticidal proteins such as *Bacillus thuringiensis* endotoxin, α -amylase inhibitor or protease inhibitors (cowpea trypsin inhibitor), glucanases, lectins (e.g. phytohemagglutinin, snowdrop lectin, wheatgerm agglutinin), RNases or ribozymes. Particular preference is given to nucleic acids which code for the chit42 endochitinase from *Trichoderma harzianum* (GenBank Acc. No.: S78423) or for the N-hydroxylating, multifunctional cytochrome P-450 (CYP79) from *Sorghum bicolor* (GenBank Acc. No.: U32624) or functional equivalents thereof.
- 10
4. Achieving defense against or attraction of insects, for example through increased release of volatile scents or messengers through, for example, enzymes of terpene biosynthesis.
 5. Achieving an ability to store in flower tissues which normally contain no storage proteins or lipids, with the aim of increasing the yield of these substances, e.g. by expression of an acetyl-CoA carboxylase or of enzymes for esterification of metabolites. Preference is given to nucleic acids which code for the *Medicago sativa* acetyl-CoA carboxylase (Accase) (GenBank Acc. No.: L25042) or functional equivalents thereof.
 - 20
 6. Expression of transport proteins which improve the uptake of metabolites, nutrients or water into the flower and thus optimize flower growth, metabolite composition or yield, for example through expression of an amino acid transporter which increases the rate of uptake of amino acids, or of a monosaccharide transporter which promotes the uptake of sugars. Preference is given to nucleic acids which code for the *Arabidopsis thaliana* cationic amino acid transporter (GenBank Acc. No.: X92657) or for the *Arabidopsis thaliana* monosaccharide transporter (Gen-Bank Acc. No.: AJ002399) or functional equivalents thereof.
 - 30
 7. Expression of genes which bring about an accumulation of fine chemicals, such as of tocopherols, tocotrienols, phenylpropanoids, isoprenoids or carotenoids, in the flower. Examples which may be mentioned are the deoxyxylulose-5-phosphate synthases, phytoene synthases, lycopene β -cyclases and the β -carotene ketolases. Preference is given to nucleic
 - 40

acids which code for the *Haematococcus pluvialis* NIES-144 (Acc. No. D45881) ketolase or functional equivalents thereof.

8. Modification of wax ester formation or of the composition of the deposited oligosaccharides to improve protection against environmental effects or to improve digestibility on use in animal or human foods. An example which may be mentioned is overexpression of endo-xyloglucan transferase. Preference is given to nucleic acids which code for the *Arabidopsis thaliana* endo-xyloglucan transferase (EXGT-A1) (Gen-Bank Acc. No. AF163819) or functional equivalents thereof.
9. Expression of genes, DNA binding proteins, dsRNA and antisense constructions for altering the flower morphology, the time of flowering and the flower senescence, and the flower metabolism. Preference is given to constructions which increase the number of petals, e.g. through down regulation of AGAMOUS and its homologous genes (Yanofsky MF et al. (1990) Nature 346:35-39), make the time of flowering earlier, e.g. through down regulation of FLOWERING LOCUS C (FLC) (Tadege M et al. (2001) Plant J 28(5):545-53) or later, e.g. through overexpression of FLC and delay senescence, e.g. through conferring a flower-specific ethylene insensitivity.
10. Generation of sterile plants by preventing pollination and/or germination by means of the expression of a suitable inhibitor, for example of a toxin, in flowers.
11. Production of nutraceuticals such as, for example,
 - a) carotenoids and/or phenylpropanoids e.g. through optimization of the flowers' own metabolic pathways, e.g. through expression of enzymes and regulators of isoprenoid biosynthesis. Preference is given to nucleic acids which code for the *Arabidopsis thaliana* chalcone synthase (GenBank Acc. No.: M20308), the *Arabidopsis thaliana* 6-4 photolyase (GenBank Acc.No.: BAB00748) or the *Arabidopsis thaliana* blue light photoreceptor/photolyase homolog (PHH1) (GenBank Acc. No.: U62549) or functional equivalents thereof. Preference is likewise given to nucleic acids which code for enzymes and regulators of isoprenoid biosynthesis such as the deoxyxylulose-5-phosphate synthases and of carotenoid biosynthesis such as the phytoene synthases, lycopene cyclases and ketolases, such as of tocopherols, tocotrienols, phenylpropanoids, isoprenoids or carotenoids, in the

flower. Examples which may be mentioned are the deoxyxylulose-5-phosphate synthases, phytoene synthases, lycopene cyclases and the carotene ketolases. Particular preference is given to nucleic acids which code for the *Haematococcus pluvialis*, NIES-144 (Acc. No. D45881) ketolase or functional equivalents.

- 10 b) polyunsaturated fatty acids such as, for example, arachidonic acid or EPA (eicosapentaenoic acid) or DHA (docosahexaenoic acid) through expression of fatty acid elongases and/or desaturases or production of proteins having improved nutritional value, such as, for example, having a high content of essential amino acids (e.g. the methionine-rich 2S albumin gene of the Brazil nut). Preference is given to nucleic acids which code for the *Bertholletia excelsa* methionine-rich 2S albumin (GenBank Acc. No.: AB044391), the *Physcomitrella patens* $\Delta 6$ -acyl lipid desaturase (GenBank Acc. No.: AJ222980; Girke et al. (1998) Plant J 15:39-48), the *Mortierella alpina* $\Delta 6$ -desaturase (Sakura-dani et al 1999 Gene 238:445-453), the 20 *Caenorhabditis elegans* $\Delta 5$ -desaturase (Michaelson et al. (1998) FEBS Letters 439:215-218), the *Caenorhabditis elegans* $\Delta 5$ -fatty-acid desaturase (des-5) (GenBank Acc. No.: AF078796), the *Mortierella alpina* $\Delta 5$ -desaturase (Michaelson et al. J Biol Chem 273:19055-19059), the *Caenorhabditis elegans* $\Delta 6$ -elongase (Beaudoin et al. (2000) Proc Natl. Acad. Sci. 97:6421-6426), the *Physcomitrella patens* $\Delta 6$ -elongase (Zank et al. (2000,) Biochemical Society Transactions 28:654-657) or functional equivalents thereof.
- 30 12. Production of pharmaceuticals such as, for example, antibodies, vaccines, hormones and/or antibiotics as described, for example, in Hood EE & Jilka JM (1999) Curr Opin Biotechnol 10(4):382-6; Ma JK & Vine ND (1999) CurrTop Microbiol Immunol 236:275-92.

Further examples of advantageous genes are mentioned for example in Dunwell JM (2000) Transgenic approaches to crop improvement. J Exp Bot. 51 Spec No:487-96.

- 40 A further aspect of the invention relates to the use of the transgenic organisms of the invention described above, and of the cells, cell cultures, parts - such as, for example, in the case of transgenic plant organisms roots, leaves etc. - and transgenic

propagation materials such as seeds or fruits, derived therefrom for producing human or animal foods, pharmaceuticals or fine chemicals.

10 Preference is further given to a method for the recombinant production of pharmaceuticals or fine chemicals in host organisms, where a host organism is transformed with one of the expression cassettes described above, and this expression cassette comprises one or more structural genes which code for the desired fine chemical, or catalyze the biosynthesis thereof, the transformed host organism is cultivated, and the desired fine chemical is isolated from the cultivation medium. This method can be applied widely to fine chemicals such as enzymes, vitamins, amino acids, sugars, fatty acids, natural and synthetic flavorings, aromatizing substances and colorants. Production of tocopherols and tocotrienols, and carotenoids such as, for example, astaxanthin is particularly preferred. Cultivation of the transformed host organisms and isolation from the host organisms or from the cultivation medium takes place by methods known to the skilled worker. The production of pharmaceuticals 20 such as, for example, antibodies or vaccines is described in Hood EE & Jilka JM (1999) Curr Opin Biotechnol 10 (4)382-6; Ma JK & Vine ND (1999) Curr Top Microbiol Immunol 236:275-92.

A further aspect of the invention relates to the use of the ϵ -cyclase promoter sequences of the invention (preferably the sequences shown in SEQ ID NO: 1, 7 or 8) for reducing the amount of protein, amount of mRNA and/or activity of an ϵ -cyclase.

Thus, when an ϵ -cyclase activity is reduced by comparison with the wild type, the amount of lycopene converted, or the amount of δ -carotene formed, in a particular time by the ϵ -cyclase protein is 30 reduced by comparison with the wild type.

"Reducing" or "reduce" is to be interpreted broadly in connection with an ϵ -cyclase or the amount of protein, amount of mRNA and/or activity, and includes the partial or substantially complete inhibition or blocking, based on various cell-biological mechanisms, of the functionality of an ϵ -cyclase in a plant cell, plant or a part, tissue, organ, cells or seeds derived therefrom.

A reduction for the purposes of the invention also includes a quantitative reduction in an ϵ -cyclase as far as substantially complete absence of the ϵ -cyclase (i.e. undetectability of 40 ϵ -cyclase activity or immunological undetectability of the

ϵ -cyclase). In this connection, a particular ϵ -cyclase (or the relevant amount of protein, amount of mRNA and/or activity) in a cell or an organism is reduced preferably by at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably 100%. Reduction means in particular also the complete absence of the ϵ -cyclase (or of its amount of protein, amount of mRNA and/or activity).

10 Various strategies for reducing the amount of protein, amount of mRNA and/or activity of the ϵ -cyclase are included according to the invention. The skilled worker will appreciate that a number of different methods are available for influencing the amount of protein, amount of mRNA and/or activity of an ϵ -cyclase in the desired way. For example, the reduction can be achieved by introducing at least one double-stranded ribonucleic acid sequence which has at least partial homology with the ϵ -cyclase promoter sequences of the invention (ϵ -cyclase promoter dsRNA). An alternative possibility is also to attach expression cassettes ensuring dsRNA expression.

20 The method of gene regulation by means of double-stranded RNA ("double-stranded RNA interference"; dsRNAi) has been described many times for animal and plant organisms (e.g. Matzke MA et al. (2000) Plant Mol Biol 43:401-415; Fire A. et al (1998) Nature 391:806-811; WO 99/32619; WO 99/53050; WO 00/68374; WO 00/44914; WO 00/44895; WO 00/49035; WO 00/63364). Reference is hereby expressly made to the processes and methods described in the indicated citations. dsRNAi methods are based on the phenomenon of simultaneous introduction of strand and complementary strand of a gene transcript bringing about a highly efficient suppression as the expression of the corresponding gene. The
30 resulting phenotype is very similar to that of a corresponding knock-out mutant (Waterhouse PM et al. (1998) Proc Natl Acad Sci USA 95:13959-64).

"Double-stranded RNA molecule" means for the purposes of the invention preferably one or more ribonucleic acid sequences which are able because of complementary sequences theoretically (e.g. according to the base-pair rules of Watson and Crick) and/or actually (e.g. on the basis of hybridization experiments in vitro and/or in vivo) to form double-stranded RNA structures. The skilled worker is aware that the formation of double-stranded RNA
40 structures represents a dynamic equilibrium. The ratio of double-stranded molecules to corresponding dissociated forms is

preferably at least 1 to 10, preferably 1:1, particularly preferably 5:1, most preferably 10:1.

A further aspect of the invention therefore relates to double-stranded RNA molecules (dsRNA molecules) which, on introduction into a plant organism (or a cell, tissue, organ or propagation material derived therefrom), bring about the reduction of at least one ϵ -cyclase. The double-stranded RNA molecule for reducing the expression of an ϵ -cyclase (ϵ -cyclase dsRNA) in this case preferably includes

- 10 a) a sense RNA strand including at least one ribonucleotide sequence which is substantially identical to at least part of a nucleic acid sequence coding for the promoter region of an ϵ -cyclase, and
- b) an antisense RNA strand which is substantially - preferably completely - complementary to the RNA sense strand under a).

The promoter region of the ϵ -cyclase is preferably described by a sequence as shown in SEQ ID NO: 1, 7 or 8.

- "Substantially identical" means that the dsRNA sequence may also have insertions, deletions and single point mutations compared
- 20 with the ϵ -cyclase promoter target sequence, and nevertheless brings about an efficient reduction of expression. The homology (as defined hereinafter) is preferably at least 75%, preferably at least 80%, very particularly preferably at least 90%, most preferably 100%, between the sense strand of an inhibitory dsRNA and at least part of the nucleic acid sequence coding for an ϵ -cyclase promoter (or between the antisense strand and the complementary strand of a nucleic acid sequence coding for an ϵ -cyclase promoter). The skilled worker moreover is aware that, in
 - 30 a comparison of homology between RNA and DNA, the bases uracil and thymine are to be regarded as equivalent.

A 100% sequence identity between dsRNA and an ϵ -cyclase promoter is not absolutely necessary for bringing about an efficient reduction of ϵ -cyclase expression. Accordingly, there is the advantage that the method is tolerant to sequence differences like those which may be present owing to genetic mutations, polymorphisms or evolutionary divergences.

The length of the partial segment is at least 10 bases, preferably at least 25 bases, particularly preferably at least 50

bases, very particularly preferably at least 100 bases, most preferably at least 200 bases or at least 300 bases.

It is alternatively possible for a "substantially identical" dsRNA also to be defined as nucleic acid sequence which is able to hybridize with part of an ϵ -cyclase gene or promoter sequence (e.g. in 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA at 50°C or 70°C for 12 to 16 h).

10 "Substantially complementary" means that the antisense RNA strand may also have insertions, deletions and single point mutations by comparison with the complement of the sense RNA strand. The homology is preferably at least 80%, preferably at least 90%, very particularly preferably at least 95%, most preferably 100%, between the antisense RNA strand and the complement of the sense RNA strand.

20 "Part of a nucleic acid sequence coding for an ϵ -cyclase promoter" means fragments of a nucleic acid sequence coding for an ϵ -cyclase promoter, preferably the promoter sequences as shown in SEQ ID NO: 1, 2 or 3 or functional equivalents thereof. In this connection, the fragments preferably have a sequence length of at least 20 bases, preferably at least 50 bases, particularly preferably at least 100 bases, very particularly preferably at least 200 bases, most preferably at least 500 bases.

It is particularly advantageous to use the ϵ -cyclase promoter region to reduce the ϵ -cyclase activity because only low homologies with other genes are present here, and thus the reduction can be highly specific without effecting the expression of other genes.

30 The dsRNA can consist of one or more strands of polyribonucleotides. It is, of course, also possible to achieve the same purpose by introducing a plurality of individual dsRNA molecules, each of which include one of the ribonucleotide sequence segments defined above, into the cell or the organism. The double-stranded dsRNA structure can be formed starting from two complementary separate RNA strands or - preferably - starting from a single self-complementary RNA strand. In this case, sense RNA strand and antisense RNA strand are preferably connected together covalently in the form of an inverted repeat.

In a preferred embodiment, a further aspect of the invention includes ribonucleic acid molecules including

- a) at least one ribonucleotide sequence which is substantially identical to at least one part of a nucleic acid sequence coding for the promoter region of an ϵ -cyclase, and
- b) at least one further ribonucleotide sequence which is substantially complementary to at least one part of the ribonucleotide sequence under a),

where a) and b) are connected together covalently, and further functional elements may be located where appropriate between a) and b).

- 10 The promoter region of the ϵ -cyclase is preferably described by a sequence as shown in SEQ ID NO: 1, 7 or 8.

As described, for example, in WO 99/53050, the dsRNA may also include a hairpin structure through connection of sense and antisense strands by a connecting sequence ("linker"; for example an intron). The self-complementary dsRNA structures are preferred, because they require merely the expression of one RNA sequence and include the complementary RNA strands always in an equimolar ratio. The connecting sequence is preferably an intron (e.g. an intron of the potato ST-LS1 gene; Vancanneyt GF et al.

- 20 (1990) Mol Gen Genet 220(2):245-250).

If the two strands of the dsRNA are to be put together in a cell or plant, this can take place in the following way, for example:

- a) transformation of the cell or plant with a vector which includes both expression cassettes,
 - b) cotransformation of the cell or plant with two vectors, where one includes the expression cassettes with the sense strand the other includes the expression cassettes with the antisense strand,
 - c) crossing of two individual plant lines, where one includes the expression cassettes with the sense strand and the other includes the expression cassettes with the antisense strand.
- 30

Formation of the RNA duplex can be initiated either outside the cell or inside it.

The dsRNA can be synthesized either in vivo or in vitro. For this purpose it is possible to put a DNA sequence coding for a dsRNA into an expression cassette under the control of at least one

genetic control element (such as, for example, a promoter). Polyadenylation is unnecessary, nor need any elements be present to initiate translation. The expression cassette for the ϵ -cyclase promoter dsRNA is preferably contained on the expression vector. The invention includes corresponding expression vectors.

10 In a particular preferred embodiment, expression of the dsRNA takes place starting from an expression construct under the functional control of a flower-specific promoter. The promoter employed in this connection is preferably not the ϵ -cyclase promoter from which the dsRNA has been derived. However, it is very possible for it to be an ϵ -cyclase promoter of a different species. Thus, for example, the sunflower ϵ -cyclase promoter could be used to express the dsRNA derived from the *Tagetes erecta* ϵ -cyclase promoter. However, expression of the dsRNA derived from an ϵ -cyclase promoter is preferably under the control of a promoter which is not an ϵ -cyclase promoter, particularly preferably under the control of the *Cucumis sativus* CHRC promoter (SEQ ID NO: 81) or of the AP3P promoter (SEQ ID NO: 77) or of a functionally equivalent part thereof.

20 The expression cassettes coding for the antisense and/or the sense strand of an ϵ -cyclase dsRNA or for the self-complementary strand of the dsRNA are for this purposes preferably inserted into a transformation vector and introduced into the plant cell using the methods described below. Stable insertion into the genome is advantageous for the method of the invention.

The dsRNA can be introduced in an amount which makes at least one copy possible per cell. Larger amounts (e.g. at least 5, 10, 100, 500 or 1000 copies per cell) may where appropriate bring about a more efficient reduction.

30 The invention also includes methods for producing ketocarotenoids, where the amount of mRNA and/or activity of at least one ϵ -cyclase is reduced by introducing at least one of the double-stranded RNA sequences or ribonucleic acid sequences of the invention or an expression cassette or expression cassettes ensuring expression thereof.

Ketocarotenoids means carotenoids which comprise at least one keto group, such as, for example, astaxanthin, canthaxanthin, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, adonirubin and adonixanthin.

Sequences

1. SEQ ID NO: 1 nucleic acid sequence coding for the *Tagetes erecta* ϵ -cyclase promoter
2. SEQ ID NO: 2 nucleic acid sequence coding for the *Tagetes erecta* ϵ -cyclase promoter including the ϵ -cyclase 5'-untranslated region
3. SEQ ID NO: 3 nucleic acid sequence coding for the *Tagetes erecta* ϵ -cyclase promoter including 5'-untranslated region and region coding for the transit peptide
4. SEQ ID NO: 4 amino acid sequence of the putative *Tagetes erecta* ϵ -cyclase transit peptide
5. SEQ ID NO: 5 nucleic acid sequence coding for the *Tagetes erecta* ϵ -cyclase promoter including the ϵ -cyclase 5'-untranslated region flanked by restriction cleavage sites for cloning
6. SEQ ID NO: 6 nucleic acid sequence coding for the *Tagetes erecta* ϵ -cyclase promoter including 5'-untranslated region and region coding for the transit peptide flanked by restriction cleavage sites for cloning
7. SEQ ID NO: 7 nucleic acid sequence coding for the *Arabidopsis thaliana* ϵ -cyclase promoter including the ϵ -cyclase 5'-untranslated region
8. SEQ ID NO: 8 nucleic acid sequence coding for the *Oryza sativa* ϵ -cyclase promoter including the ϵ -cyclase 5'-untranslated region
9. SEQ ID NO: 9 nucleic acid sequence coding for a *Tagetes erecta* ϵ -cyclase
10. SEQ ID NO: 10 amino acid sequence of a *Tagetes erecta* ϵ -cyclase
11. SEQ ID NO: 11 nucleic acid sequence coding for a *Tagetes erecta* ϵ -cyclase

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12. SEQ ID NO: 12 amino acid sequence of a *Tagetes erecta* ϵ -cyclase
 13. SEQ ID NO: 13 nucleic acid sequence coding for an *Arabidopsis thaliana* ϵ -cyclase
 14. SEQ ID NO: 14 amino acid sequence of an *Arabidopsis thaliana* ϵ -cyclase
 15. SEQ ID NO: 15 nucleic acid sequence coding for a rice ϵ -cyclase
 16. SEQ ID NO: 16 amino acid sequence of a rice ϵ -cyclase
 - 10 17.-22 SEQ ID NO: 17 to 22: sequence motifs for ϵ -cyclase proteins
 23. SEQ ID NO: 23 nucleic acid sequence coding for a *Lactuca sativa* ϵ -cyclase (homologous sequence H1)
 24. SEQ ID NO: 24 amino acid sequence of a *Lactuca sativa* ϵ -cyclase (homologous sequence H1)
 25. SEQ ID NO: 25 nucleic acid sequence coding for an *Adonis palaestina* ϵ -cyclase (homologous sequence H2)
 26. SEQ ID NO: 26 amino acid sequence of an *Adonis palaestina* ϵ -cyclase (homologous sequence H2)
 - 20 27. SEQ ID NO: 27 nucleic acid sequence coding for an *Adonis palaestina* ϵ -cyclase (homologous sequence H3)
 28. SEQ ID NO: 28 amino acid sequence of an *Adonis palaestina* ϵ -cyclase (homologous sequence H3)
 29. SEQ ID NO: 29 nucleic acid sequence coding for an *Arabidopsis thaliana* ϵ -cyclase (homologous sequence H4)
 30. SEQ ID NO: 30 amino acid sequence of an *Arabidopsis thaliana* ϵ -cyclase (homologous sequence H4)
 - 30 31. SEQ ID NO: 31 nucleic acid sequence coding for a *Citrus X paradisi* ϵ -cyclase (homologous sequence H5)
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32. SEQ ID NO: 32 amino acid sequence of Citrus X paradisi ϵ -cyclase (homologous sequence H5)
33. SEQ ID NO: 33 nucleic acid sequence coding for a Citrus X paradisi ϵ -cyclase (homologous sequence H6)
34. SEQ ID NO: 34 amino acid sequence of Citrus X paradisi ϵ -cyclase (homologous sequence H6)
35. SEQ ID NO: 35 nucleic acid sequence coding for a Citrus sinensis ϵ -cyclase (homologous sequence H7)
36. SEQ ID NO: 36 amino acid sequence of a Citrus sinensis ϵ -cyclase (homologous sequence H7)
37. SEQ ID NO: 37 nucleic acid sequence coding for a Spinacea oleracea ϵ -cyclase (homologous sequence H8)
38. SEQ ID NO: 38 amino acid sequence of a Spinacea oleracea ϵ -cyclase (homologous sequence H8)
39. SEQ ID NO: 39 nucleic acid sequence coding for a Solanum tuberosum ϵ -cyclase (homologous sequence H9)
40. SEQ ID NO: 40 amino acid sequence of a Solanum tuberosum ϵ -cyclase (homologous sequence H9)
- 20
41. SEQ ID NO: 41 nucleic acid sequence coding for a Daucus carota ϵ -cyclase (homologous sequence H10)
42. SEQ ID NO: 42 amino acid sequence of a Daucus carota ϵ -cyclase (homologous sequence H10)
43. SEQ ID NO: 43 nucleic acid sequence coding for a Daucus carota ϵ -cyclase (homologous sequence H11)
44. SEQ ID NO: 44 amino acid sequence of a Daucus carota ϵ -cyclase (homologous sequence H11)
45. SEQ ID NO: 45 nucleic acid sequence coding for a tomato ϵ -cyclase (homologous sequence H12)
- 30
46. SEQ ID NO: 46 amino acid sequence of a tomato ϵ -cyclase (homologous sequence H12)

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47. SEQ ID NO: 47 nucleic acid sequence coding for ϵ -cyclase-specific probe (gecyc1; 510 bp)
48. SEQ ID NO: 48 oligonucleotide primer PR16
5'-ggcacgaggcaaagcaaagg-3'
49. SEQ ID NO: 49 oligonucleotide primer PR22
5'-cgataagtgcgacattcaagc-3'
- 10 50. SEQ ID NO: 50 nucleic acid sequence including part of the Tagetes erecta ϵ -cyclase promoter obtained by iPCR
51. SEQ ID NO: 51 nucleic acid sequence including part of the Tagetes erecta ϵ -cyclase promoter obtained by TAIL PCR
52. SEQ ID NO: 52 oligonucleotide primer PR50
5'-cgccttgatctgtttggattgg-3'
- 20 53. SEQ ID NO: 53 oligonucleotide primer PR51
5'-ctaacaatcaatgagtatgagagc-3'
54. SEQ ID NO: 54 oligonucleotide primer PR60
5'-agagcaaggccagcaggaccacaacc-3'
55. SEQ ID NO: 55 oligonucleotide primer PR61
5'-ccttgggagcttttgggataggctag-3'
56. SEQ ID NO: 56 oligonucleotide primer PR63
5'-tcacgccttgatctgtttggattgg-3'
- 30 57. SEQ ID NO: 57 oligonucleotide primer from the set of AD1 primers as was found in the amplicon 5'-gtcgagtatggagtt-3'
58. SEQ ID NO: 58 nucleic acid sequence encoding iPCR fragment (734 bp) from pTA-ecycP
59. SEQ ID NO: 59 oligonucleotide primer OL1
5'-ctcgagagtaaaatcgttagttatg-3'
- 40 60. SEQ ID NO: 60 oligonucleotide primer OL2
5'-ccatggccattgattgtagtaattgattc-3'

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61. SEQ ID NO: 61 oligonucleotide primer OL3
5'-ccatggtaatttgcttcgtgtatctgatg-3'
62. SEQ ID NO: 62 oligonucleotide primer OL4
5'-ccatggcgctagcagcgacagtaatg-3'
63. SEQ ID NO: 63 oligonucleotide primer OL5
5'-gatatccggtgtgagggaaactag-3'
- 10 64. SEQ ID NO: 64 oligonucleotide primer PR1
5'-gcaagctcgacagctacaaacc-3'
65. SEQ ID NO: 65 oligonucleotide primer PR2
5'-gaagcatgcagctagcagcgacag-3'
66. SEQ ID NO: 66 nucleic acid sequence coding for Ketolase-
35S terminator construct
67. SEQ ID NO: 67 oligonucleotide primer PR7
5'-gagctcactc actgatttcc attgcttg-3'
- 20 68. SEQ ID NO: 68 oligonucleotide primer PR8
5'-cgccgttaagtcgatgtccgttgatttaaacagtgtc-3'
69. SEQ ID NO: 69 oligonucleotide primer PR9
5'-atcaacggac atcgacttaa cggcgtttgt aaac-3'
70. SEQ ID NO: 70 oligonucleotide primer PR10
5'-taagcttttt gttgaagaga tttgg-3'
- 30 71. SEQ ID NO: 71 oligonucleotide primer PR40
5'-gtcgactacg taagtttctg cttctacc-3'
72. SEQ ID NO: 72 oligonucleotide primer PR41
5'-ggatccggtg atacctgcac atcaac-3'
73. SEQ ID NO: 73 oligonucleotide primer PR124
5'-aagcttaccg atagtaaaat cgttagtt-3'
74. SEQ ID NO: 74 oligonucleotide primer PR125
5'-ctcgagctta ccgatagtaa aatcgtagt t-3'
- 40 75. SEQ ID NO: 75 oligonucleotide primer PR126
5'-gtcgacaaca acaacaaca acctttgc-3'

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76. SEQ ID NO: 76 oligonucleotide primer PR127
5'-ggatccaaca acaacaaaca acctttgc-3'
77. SEQ ID NO: 77 nucleic acid sequence coding for a modified version (AP3P) of the flower-specific *Arabidopsis thaliana* promoter AP3
78. SEQ ID NO: 78 nucleic acid sequence coding for PIV2 intron of the potato ST-LS1 gene.
- 10 79. SEQ ID NO: 79 nucleic acid sequence coding for the sense strand of the dsRNA directed against the ϵ -cyclase promoter
80. SEQ ID NO: 80 nucleic acid sequence coding for the antisense strand of the dsRNA directed against the ϵ -cyclase promoter
81. SEQ ID NO: 81 nucleic acid sequence coding for the *Cucumis sativus* chromoplast-specific carotenoid-associated protein (CHRC) promoter
82. SEQ ID NO: 82 oligonucleotide primer PRCHRC5
5'-gagctctaca aattagggtt ac-3'
- 20 83. SEQ ID NO: 83 oligonucleotide primer PRCHRC3
5'-aagcttatta ttccaaatt ccg-3'

Figures

The general abbreviations used in the following figures have the following meaning:

- 30 GUSI-Intron-GUSII: reporter gene (bacterial β -glucuronidase)
- Intron: Intron
- NosT: nopaline synthase (NOS) terminator sequence
- RB/LB: right or left T-DNA border
- 35-T: 35S CaMV terminator
- NptII: canamycin resistance
- NosP: nopaline synthase (NOS) promoter sequence
- aadA: bacterial spectinomycin resistance
- colE1: origin of replication

1. Fig. 1: analysis of the ϵ -cyclase transcript level total RNA isolated from leaves (L) and flower stages (1-7) of *Tagetes erecta* by means of RNA gel blotting analysis

2. Fig. 2: diagrammatic representation of the pEcycP1:GUS vector for flower-specific expression of the β -glucuronidase reporter gene (GUS) under the control of the *Tagetes erecta* ecycP1 regulatory element (promoter and 5'-untranslated region)

ecycP1: *Tagetes erecta* ϵ -cyclase promoter including
5'-untranslated region (SEQ ID NO:
2)

10. 3. Fig.3: diagrammatic representation of the pEcycP2:GUS vector for flower-specific expression of the β -glucuronidase reporter gene (GUS) under the control of the *Tagetes erecta* ecycP2 regulatory element (promoter and 5'-untranslated region and transit peptide)

ecycP2: *Tagetes erecta* ϵ -cyclase promoter including
5'-untranslated region and transit peptide
(SEQ ID NO: 3)

20. 4. Fig.4: diagrammatic representation of the pEcycP2:KETO vector for flower-specific expression of the *Haematococcus pluvialis* ketolase (KETO; SEQ ID NO: 66) under the control of the *Tagetes erecta* ecycP2 regulatory element (promoter and 5'-untranslated region and transit peptide; SEQ ID NO: 3).

5. Fig.5: diagrammatic representation of the pS5AI7 vector for flower-specific expression of ϵ -cyclase promoter specific dsRNA under the control of the AP3P promoter fragment for flower-specific reduction of the ϵ -cyclase transcript level.
AP3P: modified AP3P promoter (777 bp),
P-sense: 358 bp ϵ -cyclase promoter fragment in sense orientation,
intron: IV2 intron of the potato ST-LS1 gene
30 P-anti: the 361 bp ϵ -cyclase promoter fragment in antisense orientation.

6. Fig.6: diagrammatic representation of the pS5CI7 vector for flower-specific expression of ϵ -cyclase promoter specific dsRNA under the control of the CHRC promoter fragment for flower-specific reduction of the ϵ -cyclase transcript level

CHRC: CHRC promoter (1537 bp),
P-sense: 358 bp ϵ -cyclase promoter fragment in sense orientation,

intron: IV2 intron of the potato ST-LS1 gene

P-anti: the 361 bp ϵ -cyclase promoter fragment in antisense orientation.

7. Fig.7: iPCR amplicon comprising the 312 bp fragment of the ϵ -cyclase promoter
8. Fig.8: TAIL PCR amplicon comprising the 199 bp fragment of the ϵ -cyclase promoter
9. Fig.9: nucleotide sequence comparison between the published sequence of the *Haematococcus pluvialis* ketolase (GenBank Acc. No.: X86782) and the sequence provided within the scope of the invention (cf. example 3).
10. Fig.10: protein sequence comparison between the published sequence of the *Haematococcus pluvialis* ketolase (GenBank Acc. No.: X86782) and the sequence provided within the scope of the invention (cf. example 3).
11. Fig.11: cloning cassette for producing inverted repeat expression cassettes for flower-specific expression of ϵ -cyclase dsRNAs.
 AP3P: modified AP3P promoter (777 bp),
 rbcS: pea rbcS transit peptide (206 bp),
 intron: PIV2 intron of the ST-LS1 gene (SEQ ID NO: 78)
 term: CaMV 35S polyadenylation signal (762 bp).
12. Fig.12A-C: sequence comparison of various plant ϵ -cyclases.
 - A: GenBank Acc. No.: AF152246 (524) *Citrus x paradisi* "lycopene cyclase"
 - B: GenBank Acc. No.: AF212130 (165) *Daucus carota* partial ecyclase sequence
 - C: GenBank Acc. No.: AF229684 (201) *Daucus carota* partial ecyclase sequence
 - D: GenBank Acc. No.: AF251016 (516) *Tagetes erecta* ecyclase
 - E: GenBank Acc. No.: AF321535 (529) *Adonis palaestina* ecyclase
 - F: GenBank Acc. No.: AF321536 (529) *Adonis palaestina* ecyclase
 - G: GenBank Acc. No.: AF321537 (382) *Solanum tuberosum* partial ecyclase sequence
 - H: GenBank Acc. No.: AF321538 (533) *Lactuca sativa* ecyclase
 - I: GenBank Acc. No.: AF450280 (262) *Citrus sinensis* ecyclase

- 10 J: GenBank Acc. No.: AF463497 (517) *Spinacea oleracea* ecyclase
 K: GenBank Acc. No.: AF486650 (437) *Citrus x paradisi* ecyclase
 L: GenBank Acc. No.: AP003332 (540) rice ecyclase
 M: GenBank Acc. No.: AY099485 (525) *Tagetes erecta* ecyclase
 N: GenBank Acc. No.: L40176 (501) *Arabidopsis* "lycopene cyclase"
 O: GenBank Acc. No.: NM125085 (524) *Arabidopsis* ecyclase
 P: GenBank Acc. No.: O65837 ecyclase (526) tomato

13. Fig.13: diagrammatic representation of the inverse PCR ("iPCR")

20 For the "iPCR", genomic DNA of a target organism having the promoter sequence to be isolated is completely digested with a given restriction enzyme, and then the individual fragments are religated, i.e. connected together to form a circular molecule, in a diluted mixture. The large number of resulting circular DNA molecules includes those comprising the known sequence (i.e. the sequence coding for a homologous protein). The circular molecule can be amplified, starting therefrom, by means of PCR using a primer pair in which both primers are able to anneal to the known sequence segment.

Abbreviations: P - promoter sequence; CR - coding region; L - ligation site; PCR - polymerase chain reaction. Arrows represent the binding site of potential oligonucleotide primers in the area of the coding region.

Examples

General methods:

- 30 Oligonucleotides can be chemically synthesized for example in a known manner by the phosphoramidite method (Voet & Voet (1995), 2nd edition, Wiley Press New York, pages 896-897). The cloning steps carried out for the purposes of the present invention, such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of *E. coli* cells, culturing of bacteria, replication of phages and sequence analysis of recombinant DNA, are carried out as described in Sambrook et al. (1989) Cold Spring Harbor Laboratory Press; ISBN 0-87969-309-6.
- 40 Recombinant DNA molecules are sequenced by the method of Sanger (Sanger et al. (1977) *Proc Natl Acad Sci USA* 74:5463-5467) using an ABI laser fluorescence DNA sequencer.

Example 1: Analysis of ϵ -cyclase RNA transcript levels during the development of *Tagetes erecta* flowers

10 Total RNA from *Tagetes erecta* leaves and flowers is prepared by harvesting plant tissue, freezing it in liquid nitrogen and powdering in a mortar. 100 mg of the frozen, powdered plant tissue are then transferred into a reaction vessel and taken up in 0.8 ml of Trizol® buffer (LifeTechnologies). The suspension is extracted with 0.2 ml of chloroform. After centrifugation at 12 000 g for 15 minutes, the aqueous supernatant is removed and transferred into a new reaction vessel and extracted with one volume of ethanol. The RNA is precipitated with one volume of isopropanol and washed with 75% ethanol, and the pellet is dissolved in DEPC water (water incubated with 1/1000 volume of diethyl pyrocarbonate (DEPC) at room temperature overnight and then autoclaved). The RNA concentration is determined by photometry.

20 The relative amount of ϵ -cyclase transcript in *Tagetes* leaves and flower stages is analyzed by RNA gel blotting as described in Sambrook & Russel (2001, Molecular Cloning: A laboratory manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, Chapter 7, Protocol 6): about 10 to 15 μ g of total RNA of each sample are fractionated in a formaldehyde agarose gel. The relative amounts of total RNA can be estimated from the rRNA bands stained with ethidium bromide (Fig. 1A). The amounts of ϵ -cyclase transcript are estimated by transferring the fractionated RNA by capillary blotting to a nylon membrane.

30 A radiolabeled ϵ -cyclase-specific probe was prepared by amplifying the fragment of SEQ ID NO: 47 (gecycl) by polymerase chain reaction (PCR) from *Tagetes erecta* genomic DNA using a sense-specific primer (PR16 = 5'-ggcacgaggcaaagcaaagg-3', SEQ ID NO: 48) and an antisense-specific primer (PR22 = 5'-cgataagtgcgacattcaagc-3', SEQ ID NO: 49).

40 *Tagetes erecta* genomic DNA is prepared by harvesting leaf material from *Tagetes erecta*, freezing it in liquid nitrogen and powdering in a mortar. 100 mg of the frozen, powdered plant tissue is then transferred into a reaction vessel, taken up in 0.75 ml of extraction buffer and incubated at 65°C for 60 min. The extraction buffer is freshly prepared from 25 ml of buffer 1 (0.35 M sorbitol, 0.1 M tris base, 5 mM EDTA, pH 7.5), 25 ml of buffer 2 (0.2 M tris base, 0.05 M EDTA, 2 M NaCl, 2% CTAB), 10 ml of 5% N-lauroylsarcosine sodium) and 0.24 g of sodium bisulfite.

Incubation at 65_C is followed by mixing the suspension with 0.7 ml of chloroform/isoamyl alcohol (24:1) and then centrifuging at 10 000 g for 5 min. The upper aqueous phase is transferred into a new reaction vessel, and the chloroform/isoamyl alcohol extraction is repeated as described. The upper aqueous phase is then transferred into a new reaction vessel, and the DNA is pelleted by adding 1 ml of isopropanol and then centrifuging at 10 000 g for 5 min. The DNA pellet is washed with 0.5 ml of 75% ethanol, then dried and subsequently resuspended in 0.05 ml of sterile water by incubation at 65_C for 5 minutes.

The PCR conditions for amplification of an ϵ -cyclase-specific fragment from *Tagetes erecta* genomic DNA are as follows:

The PCR for amplifying an ϵ -cyclase-specific fragment takes place in a 50 μ l reaction mixture which contains:

- 1 μ g *Tagetes erecta* genomic DNA
- 0.25 μ M dNTPs
- 0.2 μ M primer PR16 (SEQ ID NO: 48)
- 0.2 μ M primer PR22 (SEQ ID NO: 49)
- 5 μ l 10X PCR buffer (TAKARA)
- 0.25 μ l R Taq polymerase (TAKARA)
- 25.8 μ l sterile distilled water

The PCR was carried out under the following cycle conditions: 1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute, 51_C for 2 minutes and 72_C for 3 minutes. Finally one cycle at 72_C for 10 minutes.

The PCR amplification with PR16 and PR22 results in a 510 bp fragment (SEQ ID NO: 47) which, under stringent hybridization conditions, hybridizes specifically with the ϵ -cyclase but not with the lycopene β -cyclase from *Tagetes erecta*. The amplification product is purified using the NucleoSpin[®] extract kit (Machery & Nagel) as stated by the manufacturer and employed for a radiolabeling reaction with the Highprime[®] kit (Boehringer Mannheim) as stated by the manufacturer. The prehybridization, hybridization and washing steps are carried out as described in Sambrook & Russel (2001, Molecular Cloning: A laboratory manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, Chapter 6, Protocol 10). The last washing step with 0.1 x SSC/0.1% SDS at 65_C makes the hybridization highly stringent, sufficient for specific detection with the probe described of ϵ -cyclase but not lycopene β -cyclase. The relative

ϵ -cyclase transcript levels can be estimated from the hybridization signals detected with the aid of a phosphorimager. As is evident in Fig. 1B, under the given experimental conditions, the ϵ -cyclase transcript levels in the leaves are below the limit of detection, whereas large amounts of ϵ -cyclase transcripts are detectable throughout flower development.

Example 2: Cloning of the ϵ -cyclase promoter

10 A 199 bp fragment or the 312 bp fragment of the *Tagetes erecta* ϵ -cyclase promoter can be isolated by two independent cloning strategies, inverse PCR (iPCR; adapted from Long et al. Proc Natl Acad Sci USA 90: 10370) and TAIL PCR (Liu YG et al. (1995) Plant J 8: 457-463) using genomic DNA (as described above) from the *Tagetes erecta* line Orangenprinz.

For the iPCR mixture, 2 μ g of genomic DNA are digested in a 25 μ l reaction mixture with EcoRV and RsaI, then diluted to 300 μ l and religated with 3U of ligase at 16_C overnight. PCR amplification using the primers PR50 (SEQ ID NO: 52) and PR51 (SEQ ID NO: 53) produces a fragment which comprises, in each case in sense orientation, 354 bp of the ϵ -cyclase cDNA (Genbank Acc. No.: 20 AF251016) ligated to 312 bp of the ϵ -cyclase promoter and 70 bp of the 5'-terminal region of the ϵ -cyclase cDNA (see Fig. 7).

The conditions for the PCR reactions are as follows:

The PCR for amplifying the PR50-PR51 DNA fragment which comprises inter alia the 312 bp ϵ -cyclase promoter fragment takes place in a 50 μ l reaction mixture containing:

- 1 μ l ligation mixture (prepared as described above)
- 0.25 μ M dNTPs
- 0.2 μ M primer PR50 (SEQ ID NO: 52)
- 0.2 μ M primer PR51 (SEQ ID NO: 53)
- 30 - 5 μ l 10X PCR buffer (TAKARA)
- 0.25 μ l R Taq polymerase (TAKARA)
- 28.8 μ l sterile distilled water

The PCR reactions are carried out under the following cycle conditions: 1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute, 53_C for 1 minute and 72_C for 1 minute. Finally 1 cycle at 72_C for 10 minutes.

The PCR amplification with primer PR50 and PR51 results in a 734 bp fragment which comprises inter alia the 312 bp ϵ -cyclase promoter fragment (Fig. 7). The amplicon is cloned using standard methods into the PCR cloning vector pCR2.1 (Invitrogen). Sequencings using the primers M13 and T7 result in the sequence SEQ ID NO: 50 for the amplicon.

For the TAIL PCR approach, three successive PCR reactions are carried out each with different gene-specific primers ('nested primers').

10 The TAIL1 PCR takes place in a 20 μ l reaction mixture containing:

- 100 ng genomic DNA (prepared as described above)
- 0.2 μ M each dNTP
- 0.2 μ M primer PR60 (SEQ ID NO: 54)
- 0.2 μ M AD1 primer mixture
- 2 μ l 10X PCR buffer (TAKARA)
- 0.5 U R Taq polymerase (TAKARA)
- made up to 20 μ l with sterile distilled water

The AD1 primer mixture initially represented a mixture of primers of the sequences

20 5'-(a/c/g/t)tcga(g/c)t(a/t)t(g/c)g(a/t)gtt-3'. The primer with the SEQ ID NO: 57 was found in the resulting amplicon.

The TAIL1 PCR reaction was carried out under the following cycle conditions:

- 1 cycle at 93_C for 1 minute and 95_C for 1 minute,
- 5 cycles at 94_C for 30 seconds, 62_C for 1 minute and 72_C for 2.5 minutes,
- 1 cycle at 94_C for 30 seconds, 25_C for 3 minutes, then a temperature increase to 72_C over the course of 3 minutes, 72_C for 2.5 minutes
- 30 - 15 cycles at 94_C for 10 seconds, 68_C for 1 minute and 72_C for 2.5 minutes; 94_C for 10 seconds, 68_C for 1 minute and 72_C for 2.5 minutes; 94_C for 10 seconds, 29_C for 1 minute and 72_C for 2.5 minutes;
- 1 cycle at 72_ for 5 minutes.

The TAIL2 PCR takes place in a 21 μ l reaction mixture containing;

- 1 μ l of a 1:50 dilution of the TAIL1 reaction mixture (prepared as described above)

- 0.8 μ M dNTP
- 0.2 μ M primer PR61 (SEQ ID NO: 55)
- 0.2 μ M primer AD1 (SEQ ID NO: 57)
- 2 μ l 10X PCR buffer (TAKARA)
- 0.5 U R Taq polymerase (TAKARA)
- made up to 21 μ l with sterile distilled water

The TAIL2 PCR reaction is carried out under the following cycle conditions:

- 10 - 12 cycles at 94_C for 10 seconds, 64_C for 1 minute, 72_C for 2.5 minutes, 94_C for 10 seconds, 64_C for 1 minute, 72_C for 2.5 minutes; 94_C for 10 seconds, 29_C for 1 minute, 72_C for 2.5 minutes;
- 1 cycle at 72_C for 5 minutes.

The TAIL3 PCR takes place a 100 μ l reaction mixture containing:

- 1 μ l of a 1:10 dilution of the TAIL2 reaction mixture (prepared as described above)
- 0.8 μ M dNTP
- 0.2 μ M primer PR63 (SEQ ID NO: 56)
- 0.2 μ M primer AD1 (SEQ ID NO: 57)
- 20 - 10 μ l 10X PCR buffer (TAKARA)
- 0.5 U R Taq polymerase (TAKARA)
- made up to 100 μ l with sterile distilled water

The TAIL3 PCR reaction is carried out under the following cycle conditions:

- 20 cycles at 94_C for 15 seconds, 29_C for 30 seconds, 72_C for 2 minutes;
- 1 cycle at 72_C for 5 minutes.

- 30 The PCR amplification with primer PR63 and AD1 results in a 280 bp fragment which comprises inter alia the 199 bp ϵ -cyclase promoter fragment (Fig. 8).

The amplicon was cloned using standard methods into the PCR cloning vector pCR2.1 (Invitrogen). Sequencings using the primers M13 and T7 result in the sequence SEQ ID NO: 51. This sequence is identical in the overlap region to the sequence of SEQ ID NO: 50 which is isolated using the iPCR strategy, and thus represents the nucleotide sequence in the *Tagetes erecta* line Orangenprinz used.

The pCR2.1 clone which contains the 734 bp fragment (SEQ ID NO: 58) which is isolated by the iPCR strategy is called pTA-ecycP and is used to produce the expression constructs.

Example 3: Production of transgenic ϵ -cyclase expression cassettes and expression vectors

10 The ϵ -cyclase regulatory element ecycP1 containing a promoter fragment and the 5'-untranslated region of the *Tagetes erecta* ϵ -cyclase is used to express β -glucuronidase (Jefferson et al. (1987) EMBO J 6:3901-3907) in tomato flowers (*Lycopersicon* *esculentum*). In addition, the ϵ -cyclase regulatory element ecycP2 containing a promoter fragment, the 5'-untranslated region and the putative transit peptide of the *Tagetes erecta* ϵ -cyclase is used to express either β -glucuronidase or the *Haematococcus* *pluvialis* ketolase in plastids of tomato flowers.

20 The transgenic expression vectors pEcycP1:GUS, pEcycP2:GUS, pEcycP2:KETO for the agrobacterium-mediated transformation into *Lycopersicon esculentum* were produced using the binary vector pS0301 (WO 02/00900). The transformation plasmids are produced by producing the fragments ecycP1 and ecycP2 by PCR using the clone pTA-ecycP and the primers OL1 (SEQ ID NO: 59) and OL2 (SEQ ID NO: 60) (for ecycP1) or the primers OL1 (SEQ ID NO: 59) and OL3 (SEQ ID NO: 61) (for ecycP2).

The PCR for amplifying an ϵ -cyclase-specific fragment takes place in a 50 μ l reaction mixture containing:

- 30
- 50 ng pTA-ecycP plasmid
 - 0.25 μ M dNTPs
 - 0.2 μ M primer OL1 (SEQ ID NO: 59)
 - 0.2 μ M primer OL2 (SEQ ID NO: 60) for ecycP1 or primer OL3 (SEQ ID NO: 61) for ecycP2
 - 5 μ l 10X PCR buffer (TAKARA)
 - 0.25 μ l R Taq polymerase (TAKARA)
 - 25.8 μ l sterile distilled water.

The PCR is carried out under the following cycle conditions: 1 cycle at 94_C for 2 minutes, 35 cycles at 94_C for 1 minute, 50_C for 2 minutes and 72°C for 3 minutes, finally 1 cycle at 72_C for 10 minutes.

The PCR amplification with OL1 and OL2 results in a 456 bp fragment (ecycP1, SEQ ID NO: 5), the PCR amplification with OL1

and OL3 results in a 543 bp fragment (ecycP2, SEQ ID NO: 6). The amplicons ecycP1 and ecycP2 are cloned using standard methods into the PCR cloning vector pCR2.1 (Invitrogen), and the clones pTA-ecycP1 and pTA-ecycP2 are obtained. Sequencings of the two clones confirms sequences which are identical in their respective overlap region to SEQ ID NO: 47 and SEQ ID NO: 58, respectively. These clones are therefore used for ligation into the transformation vector pS0301 (WO 02/00900).

- 10 The transformation plasmid pEcycP1:GUS is produced by isolating the 454 bp XhoI-NcoI ecycP1 fragment from pTA-ecycP1 and ligating into the XhoI-NcoI-cut vector pS0301. The clone containing the ecycP1 fragment in the correct orientation is called pEcycP1:GUS (Fig.2, construct map).

The transformation plasmid pEcycP2:GUS is produced by isolating the 541 bp XhoI-NcoI ecycP2 fragment from pTA-ecycP2 and ligating into the XhoI-NcoI-cut vector pS0301. The clone containing the ecycP2 fragment in the correct orientation is called pEcycP2:GUS (Fig.3, construct map).

- 20 The transformation plasmid pEcycP2:KETO is produced by replacing the region "GUSI/intron/GUSII/35ST" bonded by an NcoI and a HindIII restriction cleavage site in pEcycP2:GUS by a "ketolase/35S terminator" region. For this purpose, the plasmid pEcycP2:GUS is linearized by standard methods with HindIII, and the resulting 5' overhangs are filled in with Klenow fragment and finally the "GUSI/intron/GUSII/35ST" region is deleted by restriction digestion with NcoI.

The "ketolase/35S terminator" region is produced by

1. cloning a ketolase cDNA produced using RNA isolated from *Haematococcus pluvialis* (Flotow em. Wille), followed by
- 30 2. producing a transcriptional ketolase/terminator fusion by ligating the ketolase sequence into the vector pJIT117, which then serves as template for
3. the PCR amplification of the ketolase/35S terminator region.

The cDNA which codes for the *Haematococcus pluvialis* ketolase is amplified by PCR from *Haematococcus pluvialis* (strain 192.80 of the "Sammlung von Algenkulturen der Universität Göttingen") suspension culture.

To prepare total RNA from a suspension culture of *Haematococcus pluvialis* (strain 192.80) which is grown for two weeks in

indirect daylight at room temperature in *Haematococcus* medium (1.2 g/l sodium acetate, 2 g/l yeast extract, 0.2 g/l MgCl₂ x 6 H₂O, 0.02 CaCl₂ x 2 H₂O, pH 6.8; addition of 400 mg/l L-asparagine, 10 mg/l FeSO₄ x H₂O after autoclaving), the cells are harvested, frozen in liquid nitrogen and powdered in a mortar. 100 mg of the frozen, powdered algal cells are then transferred into a reaction vessel and taken up in 0.8 ml of Trizol[®] buffer (LifeTechnologies). The suspension is extracted with 0.2 ml of chloroform. After centrifugation at 12 000 g for 15 minutes, the aqueous supernatant is removed and transferred into a new reaction vessel and extracted with one volume of ethanol. The RNA is precipitated with one volume of isopropanol and washed with 75% ethanol, and the pellet is dissolved in DEPC water (water incubated with 1/1000 volume of diethyl pyrocarbonate at room temperature overnight and then autoclaved). The RNA concentration is determined by photometry.

For the cDNA synthesis, 2.5 µg of total RNA are denatured at 60°C for 10 min, cooled on ice for 2 min and transcribed into cDNA using a cDNA kit (Ready-to-go-you-prime-beads[®], Pharmacia Biotech) as stated by the manufacturer using an antisense-specific primer (PR1 SEQ ID NO: 64).

The nucleic acid encoding a ketolase from *Haematococcus pluvialis* (strain 192.80) is amplified by the polymer chain reaction (PCR) from *Haematococcus pluvialis* cDNA using a sense-specific primer (PR2; SEQ ID NO: 65) and an antisense-specific primer (PR1; SEQ ID NO: 64). The PCR conditions are as follows:

The PCR for amplifying the cDNA which codes for a ketolase protein consisting of the complete primary sequence takes place in a 50 µl reaction mixture containing:

- | | | | |
|----|---|---------|--|
| 30 | - | 4 µl | a <i>Haematococcus pluvialis</i> cDNA
(prepared as described above) |
| | - | 0.25 µM | dNTPs |
| | - | 0.2 µM | primer PR1 (SEQ ID NO: 64) |
| | - | 0.2 µM | primer PR2 (SEQ ID NO: 65) |
| | - | 5 µl | 10X PCR buffer (TAKARA) |
| | - | 0.25 µl | R Taq polymerase (TAKARA) |
| | - | 25.8 µl | sterile distilled water |

The PCR is carried out under the following cycle conditions:
1 cycle at 94°C for 2 minutes; 35 cycles at 94°C for 1 minute,

53_C for 2 minutes and 72_C for 3 minutes. Finally 1 cycle at 72_C for 10 minutes.

The PCR amplification with PR1 and PR2 results in an 1155 bp fragment which codes for a protein consisting of the complete primary sequence. Standard methods are used to clone the ketolase amplicon into the PCR cloning vector pGEM-Teasy (Promega), and the clone pGKETO2 is obtained.

10 Sequencing of the clone pGKETO2 with the T7 primer and the SP6 primer confirms a sequence which differs only in the three codons 73, 114 and 119, in one base in each case, from the published sequence (Genbank Acc. No.: X86782). These nucleotide exchanges are produced in an independent amplification experiment and thus represent the nucleotide sequence in the *Haematococcus pluvialis* strain 192.80 used (Fig. 9 and 10, sequence comparisons). This clone is used for cloning into the expression vector pJIT117 (Guerineau et al. (1988) Nucl Acids Res 16: 11380). Further cloning takes place by isolating the 1031 bp SpHI fragment from pGKETO2 and ligating into the SpHI cut vector pJIT117. The clone
20 containing the *Haematococcus pluvialis* ketolase in the correct orientation as N-terminal translational fusion with the rbcS transitpeptide is called pJKETO2.

The 1795 bp ketolase/35S terminator region is produced by PCR using pJKETO2 and the primers OL4 (SEQ ID NO: 62) and OL5 (SEQ ID NO: 63). The conditions of the PCR reactions are as follows:

The PCR for amplifying the OL4-OL5 DNA fragment which contains the coding region of the ketolase followed by the 35S terminator from CaMV takes place in a 50 μ l reaction mixture containing:

- 1 μ l pJKETO2 (1 ng of plasmid DNA)
- 0.25 μ M dNTPs
- 30 - 0.2 μ M primer OL4 (SEQ ID NO: 62)
- 0.2 μ M primer OL5 (SEQ ID NO: 63)
- 5 μ l 10X PCR buffer (TAKARA)
- 0.25 μ l R Taq polymerase (TAKARA)
- 28.8 μ l sterile distilled water

The PCR reactions are carried out under the following cycle conditions: 1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute, 53_C for 2 minutes and 72_C for 3 minutes. Finally 1 cycle at 72_C for 10 minutes.

The PCR amplification with primer OL4 and OL5 results in a 1795 bp fragment which contains the coding region of the ketolase followed by the 35S terminator from CaMV. This 1795 bp amplicon is cloned by using standard methods into the PCR cloning vector pCR2.1 (Invitrogen), and the clone "pTA-KETO/Term" is obtained. Sequencing of the clone confirms a sequence which is identical in the respective overlap region to SEQ ID NO: 66 and pJIT117. This clone is therefore used for the ligation into the transformation vector pEcycP2:GUS (see above). The transformation plasmid pEcycP2:KETO is produced by isolating the 1791 bp NcoI-EcoRV "KETO/Term" fragment from pTA-KETO/Term and ligating it into the linearized vector pEcycP2:GUS containing an NcoI-5' overhang and a blunt end. The clone containing the ecycP2 fragment in the correct orientation is called pEcycP2:KETO (Fig. 4, construct map).

Example 4: Production and analysis of transgenic tomato plants

The constructs pEcycP1:GUS, pEcycP2:GUS and pEcycP2:KETO were transformed by *Agrobacterium tumefaciens*-mediated transformation into tomato. Cotyledons and hypocotyls of seedlings seven to ten days old of the Microtom line are used as initial explant for the transformation. The culture medium of Murashige and Skoog (Murashige & Skoog (1962) *Physiol Plant* 15, 473-497) with 2% sucrose, pH 6.1, is used for germination. Germination takes place at 21_C with low light (20 to 100 μ E). After seven to ten days, the cotyledons are divided transversely, and the hypocotyls are cut into sections about 5 to 10 mm long and placed on the MSBN medium (MS, pH 6.1, 3% sucrose with 1 mg/l benzylaminopurine (BAP), 0.1 mg/l naphthaleneacetate (NAA)) which have been charged the previous day with suspension-cultivated tomato cells. The tomato cells are covered, free of air bubbles, with sterile filter paper. The explants are precultured on the described medium for three to five days. The explants are then infected with the *Agrobacterium tumefaciens* strain LBA4404, which harbors the binary plasmid with the gene to be transformed, as follows: the strain, which has been cultivated in YEB medium with the antibiotic for the binary plasmid at 28_C overnight, is centrifuged. The bacterial pellet is resuspended in liquid MS medium (3% sucrose, pH 6.1) and adjusted to an optical density of 0.3 (at 600 nm). The precultured explants are transferred into the suspension and incubated at room temperature, shaking gently, for 30 minutes. The explants are then dried with sterile filter paper and returned to their preculture medium for the three-day coculture (21_C).

After the coculture, the explants are transferred to MSZ2 medium (MS pH 6.1 with 3% sucrose, 2 mg/l zeatin, 100 mg/l kanamycin, 160 mg/l timentin) and stored for the selective regeneration at 21_C under weak light conditions (20 to 100 μ E, light/dark rhythm 16h/8h). The explants are transferred every two to three weeks until shoots form. Small shoots can be detached from the explant and rooted on MS (pH 6.1 with 3% sucrose), 160 mg/l timentin, 30 mg/l kanamycin, 0.1 mg/l IAA. Rooted plants are transferred into the glasshouse.

- 10 The transgenicity of rooted tomato plants is confirmed by PCR using genomic DNA. The activity profile of the ϵ -cyclase promoter fragment can be investigated in the case of the ecycP:GUS construct by a GUS assay by standard methods (Jefferson et al. (1987) EMBO J 6:3901-3907). The activity profile of the ϵ -cyclase promoter fragment can be investigated in the case of the pEcycP2:KETO construct by Northern blot analysis by standard methods using a ketolase-specific hybridization probe or by ketolase-specific real-time PCR (Sambrook & Russel, 2001, Molecular Cloning: A laboratory manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).
- 20

Example 5: Production of a transgenic expression vector for producing double-stranded ϵ -cyclase ribonucleic acid sequences

Expression of inverted repeat transcripts consisting of fragments of the ϵ -cyclase promoter in *Tagetes erecta* takes place under the control of a modified version (AP3P) of the flower-specific promoter AP3 from *Arabidopsis thaliana* (GenBank Acc. No.: AL132971: Nucleotide region 9298 to 10200; Hill et al. (1998) Development 125:1711-1721). The inverted repeat transcript

- 30 comprises in each case a fragment in the correct orientation (sense fragment) and a sequence-identical fragment in the contrary orientation (antisense fragment), which are connected together by a functional intron, the PIV2 intron of the potato ST-LH1 gene (Vancanneyt G et al. (1990) Mol Gen Genet 220:245-50).

- 40 The cDNA which codes for the AP3 promoter (-902 to +15) from *Arabidopsis thaliana* is produced by PCR using genomic DNA (isolated from *Arabidopsis thaliana* by a standard method) and the primers PR7 (SEQ ID NO: 67) and PR10 (SEQ ID NO: 70). The PCR conditions are as follows:

The PCR for amplifying the DNA which encodes the AP3 promoter fragment (-902 to +15) takes in place in a 50 μ l reaction mixture containing:

- 1 μ l (equivalent to 20 ng) of genomic DNA from *A. thaliana* (1:100 dil.; prepared as described above)
- 0.25 mM dNTPs
- 0.2 μ M primer PR7 (SEQ ID NO: 67)
- 0.2 μ M primer PR10 (SEQ ID NO: 70)
- 10 - 5 μ l 10X PCR buffer (Stratagene)
- 0.25 μ l Pfu polymerase (Stratagene)
- 28.8 μ l sterile distilled water.

The PCR is carried out under the following cycle conditions:
1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute, 50_C for 1 minute and 72_C for 1 minute. Finally 1 cycle at 72_C for 10 minutes.

- 20 The 922 bp amplicon is cloned using standard methods into the PCR cloning vector pCR 2.1 (Invitrogen), and the plasmid pTAP3 is obtained. Sequencing of the clone pTAP3 confirms a sequence which differs merely by an insertion (a G in position 9765 of the GenBank Acc. No.: AL132971 sequence) and a base exchange (a G in place of an A in position 9726 of the GenBank Acc. No.: AL132971 sequence) from the published AP3 sequence (GenBank Acc. No.: AL132971, nucleotide region 9298 to 10200) (position 33: T instead of G, position 55: T instead of G). These nucleotide differences can be reproduced in an independent amplification experiment and thus represent the nucleotide sequence in the *Arabidopsis thaliana* plant used.

- 30 The modified version AP3P is produced by recombinant PCR using the plasmid pTAP3. The region 10200 to 9771 is amplified using the primers PR7 (SEQ ID NO: 67) and PR9 (SEQ ID NO: 69) (amplicon A7/9), and the region 9526 to 9285 was amplified with the primers PR8 (SEQ ID NO: 68) and PR10 (SEQ ID NO: 70) (amplicon A8/10). The PCR conditions are as follows:

The PCR reaction for amplifying the DNA fragments which code for the regions 10200 to 9771 and 9526 to 9285 of the AP3 promoter takes place in 50 μ l reaction mixtures containing:

- 100 ng AP3 amplicon (described above)
- 0.25 mM dNTPs

67

- 0.2 μ M primer PR7 (SEQ ID NO: 67) or primer PR8 (SEQ ID NO: 68)
- 0.2 μ M primer PR9 (SEQ ID NO: 69) or primer PR10 (SEQ ID NO: 70)
- 5 μ l 10 X PCR buffer (Stratagene)
- 0.25 μ l Pfu Taq polymerase (Stratagene)
- 28.8 μ l sterile distilled water

10 The PCR is carried out under the following cycle conditions: 1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute, 50_C for 2 minutes and 72_C for 3 minutes. Finally 1 cycle at 72_C for 10 minutes.

The recombinant PCR comprises annealing of the amplicons A7/9 and A8/10 which overlap over a sequence of 25 nucleotides, completion of a double strand and subsequent amplification. This results in a modified version of the AP3 promoter (AP3P) in which the positions 9670 to 9526 are deleted. Denaturation (5 min at 95_C) and annealing (slow cooling at room temperature to 40_C) of the two amplicons A7/9 and A8/10 takes place in a 17.6 μ l reaction mixture containing:

- 20
- 0.5 μ g A7/9
 - 0.25 μ g A8/10

Filling-in of the 3' ends (30 min at 30°C), takes place in a 20 μ l reaction mixture containing:

- 17.6 μ l A7/9 and A8/10 annealing reaction (prepared as described above)
- 50 μ M dNTPs
- 2 μ l 1 X Klenow buffer
- 2 U Klenow enzyme

30 The nucleic acid coding for the modified promoter version AP3P is amplified by PCR using a sense-specific primer (PR7 SEQ ID NO: 67) and an antisense-specific primer (PR10 SEQ ID NO: 70). The PCR conditions are as follows:

The PCR for amplifying the AP3P fragment takes place in a 50 μ l reaction mixture containing:

- 1 μ l annealing reaction (prepared as described above)
- 0.25 mM dNTPs

68

- 0.2 μ M primer PR7 (SEQ ID NO: 67)
- 0.2 μ M primer PR10 (SEQ ID NO: 70)
- 5 μ l 10 X PCR buffer (Stratagene)
- 0.25 μ l Pfu Taq polymerase (Stratagene)
- 28.8 μ l sterile distilled water

The PCR is carried out under the following cycle conditions:
1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute,
50_C for 1 minute and 72_C for 1 minute. Finally 1 cycle at 72_C
for 10 minutes.

- 10 The PCR amplification with the primers PR7 (SEQ ID NO: 67) and PR10 (SEQ ID NO: 70) results in a 777 bp fragment which codes for the modified promoter version AP3P (SEQ ID NO: 77). The amplicon is cloned into the cloning vector pCR2.1 (Invitrogen). Sequencings with the primers T7 and M13 confirm a sequence identical to the sequence of GenBank Acc. No.: AL132971, region 10200 to 9298, the internal region from 9285 to 9526 being deleted. This clone is used for cloning into the expression vector pJIT117 (Guerineau et al. (1988) Nucl Acids Res 16:11380).

- 20 The cloning takes place by isolating the 775 bp SacI-HindIII fragment from pTAP3P and ligating into the SacI-HindIII-cut vector pJIT117. The clone which contains the promoter AP3P in place of the original promoter d35S is called pJAP3P.

A DNA fragment which comprises the PIV2 intron of the ST-LS1 gene is produced by PCR using p35SGUS INT plasmid DNA (Vancanneyt G. et al. (1990) Mol Gen Genet 220:245-250) and the primers PR40 (SEQ ID NO: 71) and PR41 (SEQ ID NO: 72). The PCR conditions are as follows:

The PCR for amplifying the sequence of the PIV2 intron of the ST-LS1 gene takes place in a 50 μ l reaction mixture containing:

- 30
- 50 ng p35SGUS INT
 - 0.25 mM dNTPs
 - 0.2 μ M primer PR40 (SEQ ID NO: 71)
 - 0.2 μ M primer PR41 (SEQ ID NO: 72)
 - 5 μ l 10X PCR buffer (TAKARA)
 - 0.25 μ l R Taq polymerase (TAKARA)
 - 28.8 μ l sterile distilled water

The PCR is carried out under the following cycle conditions:
1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute,

53_C for 1 minute and 72_C for 1 minute. Finally 1 cycle at 72_C for 10 minutes.

The PCR amplification with PR40 and PR41 results in a 212 bp fragment (SEQ ID NO: 78). The amplicon is cloned using standard methods into the PCR cloning vector pBluntII (Invitrogen), and the clone pBluntII-40-41 is obtained. Sequencing of this clone with the primer SP6 confirms a sequence which is identical to the corresponding sequence from the vector p35SGUS INT. This clone is employed for cloning into the vector pJAP3P (see above). The cloning takes place by isolating the 210 bp SalI-BamHI fragment from pBluntII-40-41 and ligating with the SalI-BamHI-cut vector pJAP3P. The clone which contains the PIV2 intron of the ST-LS1 gene in the correct orientation following the 3' end of the rbcS transit peptide is called pJAI1 and is suitable for producing expression cassettes for the flower-specific expression of inverted repeat transcripts.

Example 6: Production of inverted repeat expression cassettes for flower-specific expression of ϵ -cyclase promoter dsRNAs in *Tagetes erecta*

20

Expression of inverted repeat transcripts consisting of ϵ -cyclase promoter fragments in *Tagetes erecta* took place under the control of a modified version (AP3P) of the flower-specific promoter AP3 from *Arabidopsis* (see example 5) or of the flower-specific promoter CHRC (Genbank Acc. No. AF099501). The inverted repeat transcript contains in each case an ϵ -cyclase promoter fragment in the correct orientation (sense fragment) and a sequence-identical ϵ -cyclase promoter fragment in the contrary orientation (antisense fragment), which are connected together by a functional intron (see example 5).

30

The promoter fragments are produced by PCR using plasmid DNA (clone pTA-ecycP, see example 2) and the primers PR124 (SEQ ID NO: 73) and PR126 (SEQ ID NO: 75) or the primers PR125 (SEQ ID NO: 74) and PR127 (SEQ ID NO: 76). The conditions of the PCR reactions are as follows:

The PCR for amplifying the PR124-PR126 DNA fragment which contains the ϵ -cyclase promoter fragment takes place in a 50 μ l reaction mixture containing:

- | | |
|----|--|
| 40 | - 1 μ l pTA-ecycP (10 ng/ μ l; see example 2)
- 0.25 mM dNTPs |
|----|--|

70

- 0.2 μ M primer PR124 (SEQ ID NO: 73)
- 0.2 μ M primer PR126 (SEQ ID NO: 75)
- 5 μ l 10X PCR buffer (Stratagene)
- 0.25 μ l Pfu polymerase (Stratagene)
- 28.8 μ l sterile distilled water

The PCR for amplifying the PR125-PR127 DNA fragment containing the 312 bp ϵ -cyclase promoter fragment takes place in a 50 μ l reaction mixture containing:

- 10
- 1 μ l pTA-ecycP (10 ng/ μ l; see example 2)
 - 0.25 mM dNTPs
 - 0.2 μ M primer PR125 (SEQ ID NO: 74)
 - 0.2 μ M primer PR127 (SEQ ID NO: 76)
 - 5 μ l 10X PCR buffer (Stratagene)
 - 0.25 μ l Pfu polymerase (Stratagene)
 - 28.8 μ l sterile distilled water

The PCR reactions are carried out under the following cycle conditions: 1 cycle at 94_C for 2 minutes. 35 cycles at 94_C for 1 minute, 53_C for 1 minute and 72_C for 1 minute. Finally 1 cycle at 72_C for 10 minutes.

- 20
- The PCR amplification with primer PR124 and PR126 results in a 358 bp fragment, and PCR amplification with primer PR125 and PR127 resulted in a 361 bp fragment.

The two amplicons, the PR124-PR126 (HindIII-SalI sense) fragment and the PR125-PR127 (EcoRI-BamHI antisense) fragment, are cloned using standard methods into the PCR cloning vector pCR-BluntII (Invitrogen). Sequencings with the primer SP6 confirm in each case a sequence which, apart from the introduced restriction sites, is identical to SEQ ID NO: 58. These clones are therefore used to produce an inverted repeat construct in the cloning

30

vector pJAI1 (see example 5).

The first cloning step takes place by isolating the 356 bp PR124-PR126 HindIII-SalI fragment from the cloning vector pCR-BluntII (Invitrogen) and ligating with the HindIII-SalI-cut vector pJAI1. The clone containing the ϵ -cyclase promoter fragment in the sense orientation is called cs43. The ligation results in the sense fragment of the ϵ -cyclase promoter being inserted between the AP3P promoter and the intron. The second cloning step takes place by isolating the 359 bp PR125-PR127 BamHI-EcoRI fragment from the cloning vector pCR-BluntII (Invitrogen) and ligating with BamHI-

EcoRI-cut vector cs43. The clone containing the ϵ -cyclase promoter fragment in the antisense orientation is called cs44. The ligation results in a transcriptional fusion between the intron and the antisense fragment of the ϵ -cyclase promoter.

An inverted repeat expression cassette under the control of the CHRC promoter is produced by amplifying a CHRC promoter fragment using genomic petunia DNA (prepared by standard methods) and the primers PRCHRC5' (SEQ ID NO 82) and PRCHRC3' (SEQ ID NO: 83). The amplicon is cloned into the cloning vector pCR2.1 (Invitrogen).
 10 Sequencings of the resulting clone pCR2.1-CHRC with the primers M13 and T7 confirm a sequence identical to the GenBank Acc. No.: AF099501 sequence. This clone is therefore used for cloning into the expression vector cs44. The cloning takes place by isolating the 1535 bp SacI-HindIII fragment from pCR2.1-CHRC and ligating into the SacI-HindIII-cut vector cs44. The clone which contains the CHRC promoter in place of the original AP3P promoter is called cs45.

The transformation plasmids for the agrobacterium-mediated transformation of the AP3P-controlled inverted repeat transcript
 20 in *Tagetes erecta* are produced using the binary vector pSUN5 (WO 02/00900).

The transformation plasmid pS5AI7 is produced by ligating the 1683 bp SacI-XhoI fragment from cs44 with the SacI-XhoI-cut vector pSUN5 (Fig. 5, construct map).

The transformation plasmid pS5CI7 is produced by ligating the 2448 bp SacI-XhoI fragment from cs45 with the SacI-XhoI-cut vector pSUN5 (Fig.6, construct map).

Example 7: Production and analysis of transgenic *Tagetes* plants

The transformation plasmids pS5AI7 and pS5CI7 are transformed by
 30 *Agrobacterium tumefaciens*-mediated transformation into *Tagetes*.

Tagetes seeds are sterilized and placed on germination medium (MS medium; Murashige & Skoog (1962) *Physiol Plant* 15:473-497; pH 5.8, 2% sucrose). Germination takes place in a temperature/light/time interval of 18 to 28_C/20 to 200 μ E/3 to 16 weeks, but preferably at 21_C, 20 to 70 μ E, for 4 to 8 weeks.

All leaves of the plants which have developed in vitro by then are harvested and cut transverse to the middle. The leaf explants resulting therefrom, with a size of 10 to 60 mm², are stored

during the preparation in liquid MS medium at room temperature for not more than 2 h.

Any *Agrobacterium tumefaciens* strain, but preferably a supervirulent strain such as, for example, EHA105 with an appropriate binary plasmid, which may harbor a selection marker gene (preferably *bar* or *pat*) and one or more trait or reporter genes is cultivated overnight and used for cocultivation with the leaf material. The bacterial strain can be cultured as follows: a single colony of the appropriate strain is inoculated in YEB
10 (0.1% yeast extract, 0.5% beef extract, 0.5% peptone, 0.5% sucrose, 0.5% magnesium sulfate x 7 H₂O) with 25 mg/l kanamycin and cultured at 28°C for 16 to 20 h. The bacterial suspension is then harvested by centrifugation at 6000 g for 10 min, and resuspended in liquid MS medium so as to result in an OD₆₀₀ of about 0.1 to 0.8.

Immediately before the cocultivation, the MS medium in which the leaves have been stored is replaced by the bacterial suspension. Incubation of the leaves in the agrobacteria suspension took place at room temperature with gentle shaking for 30 min. The
20 infected explants are then put on an MS medium solidified with agar (e.g. 0.8% plant agar (Duchefa, NL)), with growth regulators such as, for example, 3 mg/l benzylaminopurine (BAP) and 1 mg/l indolylacetic acid (IAA). The orientation of the leaves on the medium is immaterial. Cultivation of the explants takes place for 1 to 8 days, but preferably for 6 days, during which the following conditions can be used: light intensity: 30 to 80 $\mu\text{mol/m}^2 \times \text{sec}$, temperature: 22 to 24°C, 16/18 hours light/dark alternation. The cocultivated explants are then transferred to fresh MS medium, preferably with the same growth regulators, this
30 second medium additionally containing an antibiotic to suppress bacterial growth. Timentin in a concentration of 200 to 500 mg/l is very suitable for this purpose. The second selective component employed is one for selecting for successful transformation. Phosphinothricin in a concentration of 1 to 5 mg/l selects very efficiently, but other selective components according to the method to be used are also conceivable. After one to three weeks in each case, the explants are transferred to fresh medium until plumules and small shoots develop, which are then transferred to the same basal medium including timentin and PPT or alternative
40 components with growth regulators, namely, for example, 0.5 mg/l indolylbutyric acid (IBA) and 0.5 mg/l gibberillic acid GA₃, for rooting. Rooted shoots can be transferred into the glasshouse.

In addition to the method described, the following advantageous modifications are possible:

- before the explants are infected with the bacteria, they can be preincubated on the medium described above for the cocultivation for 1 to 12 days, preferably 3 to 4. This is followed by infection, cocultivation and selection regeneration as described above.
 - the pH for the regeneration (normally 5.8) can be lowered to pH 5.2. This improves control of the growth of agrobacteria.
- 10 - addition of AgNO₃ (3 to 10 mg/l) to the regeneration medium improves the condition of the culture, including the regeneration itself.
- components which reduce phenol formation and are known to the skilled worker, such as, for example citric acid, ascorbic acid, PVP and many others, have beneficial effects on the culture.
 - liquid culture medium can also be used for the whole method. The culture can also be incubated on commercially available supports which are positioned on the liquid medium.
- 20 The transgenicity of rooted shoots can be investigated on isolated genomic DNA by the polymerase chain reaction (PCR). The reduction in the amounts of ϵ -cyclase transcript (compared with the wild type used for the transformation) as a result of transformation with the transformation plasmid pS5AI7 or pS5CI7 can be investigated by Northern gel blot analysis by standard methods (Sambrook & Russel, 2001, Molecular Cloning: A laboratory manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) using an ϵ -cyclase-specific hybridization probe, for example produced as described in example 1. In
- 30 addition, the reduction in the amounts of ϵ -cyclase transcript (compared with the wild type used for the transformation) can be investigated by ϵ -cyclase-specific real time PCR.
-

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in the flower of plants

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taggtcgcta cgttttcctaa cttgtcgctt attaggctac ttttaaactc cacgcaccaa 720
tccagttctt acacgcgctt tcacaaaaag gaccttacat gggttgttcc cccaataaga 780
aaattacacg ttgcagaatc gaagcagtggt tctatccttg ttatcgctct ctcgtcttta 840
tggctcttcc aagtcatttt gagaaatttt cgatttttag atttttttcc aaatattaca 900
aaaggaaata attagattcc tctttctgct tgctatacct tgatagaaca atataacaat 960
ggtgtaagtc ttctcgctgt attcgaaatt atttgaggga ggaaaatg 1008

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<210> 8
<211> 1169
<212> DNA
<213> *Oryza sativa*

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gggaagcagc ggacgatgcc gagcaggaca atgtcctcct cgtcaccacc ttctcatcgc 180
tcgtcgcggt cgggtgggtgc cgggcgggag ggtttttgag ggaggggtcg gcggccggcg 240
agcccatccg agctcgccgt gcttgcgcga gcccgccctc gctgcagctt ggtgtgctgg 300
ggacgacgac gaggaaggag ggagagagga atcaactacc gacgacagcg gaggcggcg 360
agcgcggtgc cgggctgctc acgctcgccg accaggccct cgctccgtea cactcgccctg 420
cctccgttcc acccgcgacc cgcgcgcctc cgttcgcage gctcgcccgt cgccgctccg 480
ctgctgtgca gtcccgcgcg cgcgctcgcc ctgactgaag aagaaaagag agaagagaga 540
aaagagaagg gaaggagaag aaaatagaag aaaaaaatat gtgcagctga tgtatgagcc 600
ccacatactc ttttttaatc ttttttgctg actacgatgc cacgtcagcg aaaccaccta 660
tatatactac cataggatct tgagtgcacg gtttatatga gtttaggagt atacattttt 720
agttttatgg ttaagggatc ataaaaaatt ctgcctatta agttgagtga cgcgcagtga 780
acttattact caaacttaac agcgtttgat ccattcacat ccggcccata gcagcccata 840
tccagcaacg cagcacgggt actgacacgc ggggcccac gaggtggcgc cgtggctggg 900
ggggcggggtg agtcacgccc tcggcgctgc ccccaaaagc gaaccatata ctttgcttcg 960
tggggccctg cccaatcgcc gcccgccacg tgccccttcc cggataccgc ctccctccaa 1020
acccgctccc tctccctctc cctcctccta caatggccgc agcagcagca gccagcagca 1080
gacgcagaga ccagtagctt tcgcagaggc ggcagccacc accgcctcct cctcctcctc 1140
ctcatccgac ggtgtgcaac caagcgatg 1169

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<210> 9
<211> 1830
<212> DNA
<213> *Tagetes erecta*

<220>
<221> CDS
<222> (141)..(1688)
<223> coding for epsilon-cyclase

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gatacaaggc gtgactggat atttctctct cgttcctaac aacagcaacg aagaagaaaa 120
agaatcatta ctaacaatca atg agt atg aga gct gga cac atg acg gca aca 173
Met Ser Met Arg Ala Gly His Met Thr Ala Thr

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atg gcg gct ttt aca tgc cct agg ttt atg act agc atc aga tac acg	221
Met Ala Ala Phe Thr Cys Pro Arg Phe Met Thr Ser Ile Arg Tyr Thr	
15 20 25	
aag caa att aag tgc aac gct gct aaa agc cag cta gtc gtt aaa caa	269
Lys Gln Ile Lys Cys Asn Ala Ala Lys Ser Gln Leu Val Val Lys Gln	
30 35 40	
gag att gag gag gaa gaa gat tat gtg aaa gcc ggt gga tcg gag ctg	317
Glu Ile Glu Glu Glu Glu Asp Tyr Val Lys Ala Gly Gly Ser Glu Leu	
45 50 55	
ctt ttt gtt caa atg caa cag aat aag tcc atg gat gca cag tct agc	365
Leu Phe Val Gln Met Gln Gln Asn Lys Ser Met Asp Ala Gln Ser Ser	
60 65 70 75	
cta tcc caa aag ctc cca agg gta cca ata gga gga gga gga gac agt	413
Leu Ser Gln Lys Leu Pro Arg Val Pro Ile Gly Gly Gly Gly Asp Ser	
80 85 90	
aac tgt ata ctg gat ttg gtt gta att ggt tgt ggt cct gct ggc ctt	461
Asn Cys Ile Leu Asp Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu	
95 100 105	
gct ctt gct gga gaa tca gcc aag cta ggc ttg aat gtc gca ctt atc	509
Ala Leu Ala Gly Glu Ser Ala Lys Leu Gly Leu Asn Val Ala Leu Ile	
110 115 120	
ggc cct gat ctt cct ttt aca aat aac tat ggt gtt tgg gag gat gaa	557
Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu	
125 130 135	
ttt ata ggt ctt gga ctt gag ggc tgt att gaa cat gtt tgg cga gat	605
Phe Ile Gly Leu Gly Leu Glu Gly Cys Ile Glu His Val Trp Arg Asp	
140 145 150 155	
act gta gta tat ctt gat gac aac gat ccc att ctc ata ggt cgt gcc	653
Thr Val Val Tyr Leu Asp Asp Asn Asp Pro Ile Leu Ile Gly Arg Ala	
160 165 170	
tat gga cga gtt agt cgt gat tta ctt cac gag gag ttg ttg act agg	701
Tyr Gly Arg Val Ser Arg Asp Leu Leu His Glu Glu Leu Leu Thr Arg	
175 180 185	
tgc atg gag tca ggc gtt tca tat ctg agc tcc aaa gtg gaa cgg att	749
Cys Met Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile	
190 195 200	
act gaa gct cca aat ggc cta agt ctc ata gag tgt gaa ggc aat atc	797
Thr Glu Ala Pro Asn Gly Leu Ser Leu Ile Glu Cys Glu Gly Asn Ile	
205 210 215	
aca att cca tgc agg ctt gct act gtc gct tct gga gca gct tct gga	845
Thr Ile Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly	
220 225 230 235	
aaa ctt ttg cag tat gaa ctt ggc ggt ccc cgt gtt tgc gtt caa aca	893
Lys Leu Leu Gln Tyr Glu Leu Gly Gly Pro Arg Val Cys Val Gln Thr	
240 245 250	
gct tat ggt ata gag gtt gag gtt gaa agc ata ccc tat gat cca agc	941
Ala Tyr Gly Ile Glu Val Glu Val Glu Ser Ile Pro Tyr Asp Pro Ser	
255 260 265	

cta atg gtt ttc atg gat tat aga gac tac acc aaa cat aaa tct caa	989
Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Thr Lys His Lys Ser Gln	
270 275 280	
tca cta gaa gca caa tat cca aca ttt ttg tat gtc atg cca atg tct	1037
Ser Leu Glu Ala Gln Tyr Pro Thr Phe Leu Tyr Val Met Pro Met Ser	
285 290 295	
cca act aaa gta ttc ttt gag gaa act tgt ttg gct tca aaa gag gcc	1085
Pro Thr Lys Val Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Glu Ala	
300 305 310 315	
atg cct ttt gag tta ttg aag aca aaa ctc atg tca aga tta aag act	1133
Met Pro Phe Glu Leu Leu Lys Thr Lys Leu Met Ser Arg Leu Lys Thr	
320 325 330	
atg ggg atc cga ata acc aaa act tat gaa gag gaa tgg tca tat att	1181
Met Gly Ile Arg Ile Thr Lys Thr Tyr Glu Glu Glu Trp Ser Tyr Ile	
335 340 345	
cca gta ggt gga tcc tta cca aat acc gag caa aag aac ctt gca ttt	1229
Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys Asn Leu Ala Phe	
350 355 360	
ggg gct gct gct agc atg gtg cat cca gcc aca gga tat tgg gtt gta	1277
Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val	
365 370 375	
aga tca ctg tca gaa gct cct aat tat gca gca gta att gca aag att	1325
Arg Ser Leu Ser Glu Ala Pro Asn Tyr Ala Ala Val Ile Ala Lys Ile	
380 385 390 395	
tta ggg aaa gga aat tca aaa cag atg ctt gat cat gga aga tac aca	1373
Leu Gly Lys Gly Asn Ser Lys Gln Met Leu Asp His Gly Arg Tyr Thr	
400 405 410	
acc aac atc tca aag caa gct tgg gaa aca ctt tgg ccc ctt gaa agg	1421
Thr Asn Ile Ser Lys Gln Ala Trp Glu Thr Leu Trp Pro Leu Glu Arg	
415 420 425	
aaa aga cag aga gca ttc ttt ctc ttt gga tta gca ctg att gtc cag	1469
Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu Ile Val Gln	
430 435 440	
atg gat att gag ggg acc cgc aca ttc ttc cgg act ttc ttc cgc ttg	1517
Met Asp Ile Glu Gly Thr Arg Thr Phe Phe Arg Thr Phe Phe Arg Leu	
445 450 455	
ccc aca tgg atg tgg tgg ggg ttt ctt gga tct tgg tta tca tca act	1565
Pro Thr Trp Met Trp Trp Gly Phe Leu Gly Ser Ser Leu Ser Ser Thr	
460 465 470 475	
gac ttg ata ata ttt gcg ttt tac atg ttt atc ata gca ccg cat agc	1613
Asp Leu Ile Ile Phe Ala Phe Tyr Met Phe Ile Ile Ala Pro His Ser	
480 485 490	
ctg aga atg ggt ctg gtt aga cat ttg ctt tct gac ccg aca gga gga	1661
Leu Arg Met Gly Leu Val Arg His Leu Leu Ser Asp Pro Thr Gly Gly	
495 500 505	
aca atg tta aaa gcg tat ctc acg ata taaataactc tagtcgcgat	1708
Thr Met Leu Lys Ala Tyr Leu Thr Ile	
510 515	
cagtttagat tataggcaca tcttgcatat atatatgtat aaaccttatg tgtgctgtat	1768
ccttacatca acacagtcac taattgtatt tcttggggta atgctgatga agtattttct	1828

99

1830

<210> 10

<211> 516

<212> PRT

<213> Tagetes erecta

<400> 10

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Cys Pro Arg Phe Met Thr Ser Ile Arg Tyr Thr Lys Gln Ile Lys Cys
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Asn Ala Ala Lys Ser Gln Leu Val Val Lys Gln Glu Ile Glu Glu Glu
35 40 45
Glu Asp Tyr Val Lys Ala Gly Gly Ser Glu Leu Leu Phe Val Gln Met
50 55 60
Gln Gln Asn Lys Ser Met Asp Ala Gln Ser Ser Leu Ser Gln Lys Leu
65 70 75 80
Pro Arg Val Pro Ile Gly Gly Gly Gly Asp Ser Asn Cys Ile Leu Asp
85 90 95
Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Gly Glu
100 105 110
Ser Ala Lys Leu Gly Leu Asn Val Ala Leu Ile Gly Pro Asp Leu Pro
115 120 125
Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Ile Gly Leu Gly
130 135 140
Leu Glu Gly Cys Ile Glu His Val Trp Arg Asp Thr Val Val Tyr Leu
145 150 155 160
Asp Asp Asn Asp Pro Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser
165 170 175
Arg Asp Leu Leu His Glu Glu Leu Leu Thr Arg Cys Met Glu Ser Gly
180 185 190
Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala Pro Asn
195 200 205
Gly Leu Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro Cys Arg
210 215 220
Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr
225 230 235 240
Glu Leu Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Ile Glu
245 250 255
Val Glu Val Glu Ser Ile Pro Tyr Asp Pro Ser Leu Met Val Phe Met
260 265 270
Asp Tyr Arg Asp Tyr Thr Lys His Lys Ser Gln Ser Leu Glu Ala Gln
275 280 285
Tyr Pro Thr Phe Leu Tyr Val Met Pro Met Ser Pro Thr Lys Val Phe
290 295 300
Phe Glu Glu Thr Cys Leu Ala Ser Lys Glu Ala Met Pro Phe Glu Leu
305 310 315 320
Leu Lys Thr Lys Leu Met Ser Arg Leu Lys Thr Met Gly Ile Arg Ile
325 330 335

Thr Lys Thr Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser
340 345 350
Leu Pro Asn Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser
355 360 365
Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu
370 375 380
Ala Pro Asn Tyr Ala Ala Val Ile Ala Lys Ile Leu Gly Lys Gly Asn
385 390 395 400
Ser Lys Gln Met Leu Asp His Gly Arg Tyr Thr Thr Asn Ile Ser Lys
405 410 415
Gln Ala Trp Glu Thr Leu Trp Pro Leu Glu Arg Lys Arg Gln Arg Ala
420 425 430
Phe Phe Leu Phe Gly Leu Ala Leu Ile Val Gln Met Asp Ile Glu Gly
435 440 445
Thr Arg Thr Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp
450 455 460
Trp Gly Phe Leu Gly Ser Ser Leu Ser Ser Thr Asp Leu Ile Ile Phe
465 470 475 480
Ala Phe Tyr Met Phe Ile Ile Ala Pro His Ser Leu Arg Met Gly Leu
485 490 495
Val Arg His Leu Leu Ser Asp Pro Thr Gly Gly Thr Met Leu Lys Ala
500 505 510
Tyr Leu Thr Ile
515

<210> 11
<211> 1916
<212> DNA
<213> Tagetes erecta
<220>
<221> CDS
<222> (173)..(1747)
<223> coding for epsilon-cyclase

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tcgttcctta acaacagcaa cgaagaagaa aaagaatcat tactcacaat ca atg agt 178
Met Ser
1
atg aga gct gga cac atg acg gca aca atg gcg gct ttt aca tgc cct 226
Met Arg Ala Gly His Met Thr Ala Thr Met Ala Ala Phe Thr Cys Pro
5 10 15
agg ttt atg act agc atc aga tac acg aag caa att aag tgc aac gct 274
Arg Phe Met Thr Ser Ile Arg Tyr Thr Lys Gln Ile Lys Cys Asn Ala
20 25 30
gct aaa agc cag cta gtc gtt aaa caa gag att gag gag gaa gaa gat 322
Ala Lys Ser Gln Leu Val Val Lys Gln Glu Ile Glu Glu Glu Glu Asp
35 40 45 50

tat gtg aaa gcc ggt gga tgc gag ctg ctt ttt gtt caa atg caa cag	370
Tyr Val Lys Ala Gly Gly Ser Glu Leu Leu Phe Val Gln Met Gln Gln	
55 60 65	
aat aag tcc atg gat gca cag tct agc cta tcc caa aag ctc cca agg	418
Asn Lys Ser Met Asp Ala Gln Ser Ser Leu Ser Gln Lys Leu Pro Arg	
70 75 80	
gta cca ata gga gga gga gga gac agt aac tgt ata ctg gat ttg gtt	466
Val Pro Ile Gly Gly Gly Gly Asp Ser Asn Cys Ile Leu Asp Leu Val	
85 90 95	
gta att ggt tgt ggt cct gct ggc ctt gct ctt gct gga gaa tca gcc	514
Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Gly Glu Ser Ala	
100 105 110	
aag cta ggc ttg aat gtc gca ctt atc ggc cct gat ctt cct ttt aca	562
Lys Leu Gly Leu Asn Val Ala Leu Ile Gly Pro Asp Leu Pro Phe Thr	
115 120 125 130	
aat aac tat ggt gtt tgg gag gat gaa ttt ata ggt ctt gga ctt gag	610
Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Ile Gly Leu Gly Leu Glu	
135 140 145	
ggc tgt att gaa cat gtt tgg cga gat act gta gta tat ctt gat gac	658
Gly Cys Ile Glu His Val Trp Arg Asp Thr Val Val Tyr Leu Asp Asp	
150 155 160	
aac gat ccc att ctc ata ggt cgt gcc tat gga cga gtt agt cgt gat	706
Asn Asp Pro Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg Asp	
165 170 175	
tta ctt cac gag gag ttg ttg act agg tgc atg gag tca ggc gtt tca	754
Leu Leu His Glu Glu Leu Leu Thr Arg Cys Met Glu Ser Gly Val Ser	
180 185 190	
tat ctg agc tcc aaa gtg gaa cgg att act gaa gct cca aat ggc cta	802
Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala Pro Asn Gly Leu	
195 200 205 210	
agt ctc ata gag tgt gaa ggc aat atc aca att cca tgc agg ctt gct	850
Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro Cys Arg Leu Ala	
215 220 225	
act gtc gct tct gga gca gct tct ggg aaa ctt ttg cag tat gaa ctt	898
Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Leu	
230 235 240	
ggc ggt ccc cgt gtt tgc gtt caa aca gct tat ggt tac gag gtt gag	946
Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Tyr Glu Val Glu	
245 250 255	
gtt gaa agc ata ccc tat gat cca agc cta atg gtt ttc atg gat tat	994
Val Glu Ser Ile Pro Tyr Asp Pro Ser Leu Met Val Phe Met Asp Tyr	
260 265 270	
aga gac tac acc aaa cat aaa tct caa tca cta gaa gca caa tat cca	1042
Arg Asp Tyr Thr Lys His Lys Ser Gln Ser Leu Glu Ala Gln Tyr Pro	
275 280 285 290	
aca ttt ttg tat gtc atg cca atg tct cca act aaa gta ttc ttt gag	1090
Thr Phe Leu Tyr Val Met Pro Met Ser Pro Thr Lys Val Phe Glu	
295 300 305	

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gaa act tgt ttg gct tca aaa gag gcc atg cct ttt gag tta ttg aag 1138
Glu Thr Cys Leu Ala Ser Lys Glu Ala Met Pro Phe Glu Leu Leu Lys
      310                      315                      320

aca aaa ctc atg tca aga tta aag act atg ggg atc cga ata acc aaa 1186
Thr Lys Leu Met Ser Arg Leu Lys Thr Met Gly Ile Arg Ile Thr Lys
      325                      330                      335

act tat gaa gag tat ctt gtt gct tgt caa tat ttg gaa gaa tgg tca 1234
Thr Tyr Glu Glu Tyr Leu Val Ala Cys Gln Tyr Leu Glu Glu Trp Ser
      340                      345                      350

tat att cca gta ggt gga tcc ctt cca aat acc gag caa aag aac ctt 1282
Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys Asn Leu
      355                      360                      365                      370

gca ttt ggt gct gct gct agc atg gtg cat cca gcc aca gga tat tgc 1330
Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser
      375                      380                      385

gtt gta aga tca ctg tca gaa gct cct aat tat gca gca gta att gca 1378
Val Val Arg Ser Leu Ser Glu Ala Pro Asn Tyr Ala Ala Val Ile Ala
      390                      395                      400

aag att tta ggg aaa gga aat tca aaa cag atg ctt gat ctt gga aga 1426
Lys Ile Leu Gly Lys Gly Asn Ser Lys Gln Met Leu Asp Leu Gly Arg
      405                      410                      415

tac aca acc aac atc tca aag caa gct tgg gaa aca ctt tgg ccc ctt 1474
Tyr Thr Thr Asn Ile Ser Lys Gln Ala Trp Glu Thr Leu Trp Pro Leu
      420                      425                      430

gaa agg aaa aga cag aga gca ttc ttt ctc ttt gga tta gca ctg att 1522
Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu Ile
      435                      440                      445                      450

gtc cag atg gat att gag ggg acc cgc aca ttc ttc cgg act ttc ttc 1570
Val Gln Met Asp Ile Glu Gly Thr Arg Thr Phe Phe Arg Thr Phe Phe
      455                      460                      465

cgc ttg ccc aca tgg atg tgg tgg ggg ttt ctt gga tct tgc tta tca 1618
Arg Leu Pro Thr Trp Met Trp Trp Gly Phe Leu Gly Ser Ser Leu Ser
      470                      475                      480

tca act gac ttg ata ata ttt gcg ttt tac atg ttt atc ata gca ccg 1666
Ser Thr Asp Leu Ile Ile Phe Ala Phe Tyr Met Phe Ile Ile Ala Pro
      485                      490                      495

cat agc ctg aga atg ggt ctg gtt aga cat ttg ctt tct gac ccg aca 1714
His Ser Leu Arg Met Gly Leu Val Arg His Leu Leu Ser Asp Pro Thr
      500                      505                      510

gga gga aca atg tta aaa gcg tat ctc acg ata taaataactc tagtcgcgat 1767
Gly Gly Thr Met Leu Lys Ala Tyr Leu Thr Ile
      515                      520                      525

cagtttagat tataggcaca tcttgcatat atatattgtat aaaccttatg tgtgctgtat 1827
ccttacatca acacagtcac taattgtatt tcttggggta atgctgatga agtattttct 1887
ggaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1916

<210> 12
<211> 525
<212> PRT
<213> Tagetes erecta

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<400> 12
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Cys Pro Arg Phe Met Thr Ser Ile Arg Tyr Thr Lys Gln Ile Lys Cys
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35 40 45
Glu Asp Tyr Val Lys Ala Gly Gly Ser Glu Leu Leu Phe Val Gln Met
50 55 60
Gln Gln Asn Lys Ser Met Asp Ala Gln Ser Ser Leu Ser Gln Lys Leu
65 70 75 80
Pro Arg Val Pro Ile Gly Gly Gly Gly Asp Ser Asn Cys Ile Leu Asp
85 90 95
Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Gly Glu
100 105 110
Ser Ala Lys Leu Gly Leu Asn Val Ala Leu Ile Gly Pro Asp Leu Pro
115 120 125
Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Ile Gly Leu Gly
130 135 140
Leu Glu Gly Cys Ile Glu His Val Trp Arg Asp Thr Val Val Tyr Leu
145 150 155 160
Asp Asp Asn Asp Pro Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser
165 170 175
Arg Asp Leu Leu His Glu Glu Leu Leu Thr Arg Cys Met Glu Ser Gly
180 185 190
Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala Pro Asn
195 200 205
Gly Leu Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro Cys Arg
210 215 220
Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr
225 230 235 240
Glu Leu Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Tyr Glu
245 250 255
Val Glu Val Glu Ser Ile Pro Tyr Asp Pro Ser Leu Met Val Phe Met
260 265 270
Asp Tyr Arg Asp Tyr Thr Lys His Lys Ser Gln Ser Leu Glu Ala Gln
275 280 285
Tyr Pro Thr Phe Leu Tyr Val Met Pro Met Ser Pro Thr Lys Val Phe
290 295 300
Phe Glu Glu Thr Cys Leu Ala Ser Lys Glu Ala Met Pro Phe Glu Leu
305 310 315 320
Leu Lys Thr Lys Leu Met Ser Arg Leu Lys Thr Met Gly Ile Arg Ile
325 330 335
Thr Lys Thr Tyr Glu Glu Tyr Leu Val Ala Cys Gln Tyr Leu Glu Glu
340 345 350
Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys
355 360 365

Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly
370 375 380
Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Asn Tyr Ala Ala Val
385 390 395 400
Ile Ala Lys Ile Leu Gly Lys Gly Asn Ser Lys Gln Met Leu Asp Leu
405 410 415
Gly Arg Tyr Thr Thr Asn Ile Ser Lys Gln Ala Trp Glu Thr Leu Trp
420 425 430
Pro Leu Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala
435 440 445
Leu Ile Val Gln Met Asp Ile Glu Gly Thr Arg Thr Phe Phe Arg Thr
450 455 460
Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe Leu Gly Ser Ser
465 470 475 480
Leu Ser Ser Thr Asp Leu Ile Ile Phe Ala Phe Tyr Met Phe Ile Ile
485 490 495
Ala Pro His Ser Leu Arg Met Gly Leu Val Arg His Leu Leu Ser Asp
500 505 510
Pro Thr Gly Gly Thr Met Leu Lys Ala Tyr Leu Thr Ile
515 520 525

<210> 13
<211> 1818
<212> DNA
<213> Arabidopsis thaliana
<220>
<221> CDS
<222> (104)..(1675)
<223> coding for epsilon-cyclase
<400> 13

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tgtaagtctt ctcgctgtat tcgaaattat ttggaggagg aaa atg gag tgt gtt 115
Met Glu Cys Val
1
ggg gct agg aat ttc gca gca atg gcg gtt tca aca ttt ccg tca tgg 163
Gly Ala Arg Asn Phe Ala Ala Met Ala Val Ser Thr Phe Pro Ser Trp
5 10 15 20
agt tgt cga agg aaa ttt cca gtg gtt aag aga tac agc tat agg aat 211
Ser Cys Arg Arg Lys Phe Pro Val Val Lys Arg Tyr Ser Tyr Arg Asn
25 30 35
att cgt ttc ggt ttg tgt agt gtc aga gct agc ggc ggc gga agt tcc 259
Ile Arg Phe Gly Leu Cys Ser Val Arg Ala Ser Gly Gly Gly Ser Ser
40 45 50
ggg agt gag agt tgt gta gcg gtg aga gaa gat ttc gct gac gaa gaa 307
Gly Ser Glu Ser Cys Val Ala Val Arg Glu Asp Phe Ala Asp Glu Glu
55 60 65
gat ttt gtg aaa gct ggt ggt tct gag att cta ttt gtt caa atg cag 355
Asp Phe Val Lys Ala Gly Gly Ser Glu Ile Leu Phe Val Gln Met Gln
70 75 80

cag aac aaa gat atg gat gaa cag tct aag ctt gtt gat aag ttg cct	403
Gln Asn Lys Asp Met Asp Glu Gln Ser Lys Leu Val Asp Lys Leu Pro	
85 90 95 100	
cct ata tca att ggt gat ggt gct ttg gat cta gtg gtt att ggt tgt	451
Pro Ile Ser Ile Gly Asp Gly Ala Leu Asp Leu Val Val Ile Gly Cys	
105 110 115	
ggt cct gct ggt tta gcc ttg gct gca gaa tca gct aag ctt gga tta	499
Gly Pro Ala Gly Leu Ala Leu Ala Glu Ser Ala Lys Leu Gly Leu	
120 125 130	
aaa gtt gga ctc att ggt cca gat ctt cct ttt act aac aat tac ggt	547
Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly	
135 140 145	
gtt tgg gaa gat gaa ttc aat gat ctt ggg ctg caa aaa tgt att gag	595
Val Trp Glu Asp Glu Phe Asn Asp Leu Gly Leu Gln Lys Cys Ile Glu	
150 155 160	
cat gtt tgg aga gag act att gtg tat ctg gat gat gac aag cct att	643
His Val Trp Arg Glu Thr Ile Val Tyr Leu Asp Asp Asp Lys Pro Ile	
165 170 175 180	
acc att ggc cgt gct tat gga aga gtt agt cga cgt ttg ctc cat gag	691
Thr Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg Arg Leu Leu His Glu	
185 190 195	
gag ctt ttg agg agg tgt gtc gag tca ggt gtc tcg tac ctt agc tcg	739
Glu Leu Leu Arg Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser	
200 205 210	
aaa gtt gac agc ata aca gaa gct tct gat ggc ctt aga ctt gtt gct	787
Lys Val Asp Ser Ile Thr Glu Ala Ser Asp Gly Leu Arg Leu Val Ala	
215 220 225	
tgt gac gac aat aac gtc att ccc tgc agg ctt gcc act gtt gct tct	835
Cys Asp Asp Asn Asn Val Ile Pro Cys Arg Leu Ala Thr Val Ala Ser	
230 235 240	
gga gca gct tcg gga aag ctc ttg caa tac gaa gtt ggt gga cct aga	883
Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val Gly Gly Pro Arg	
245 250 255 260	
gtc tgt gtg caa act gca tac ggc gtg gag gtt gag gtg gaa aat agt	931
Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Ser	
265 270 275	
cca tat gat cca gat caa atg gtt ttc atg gat tac aga gat tat act	979
Pro Tyr Asp Pro Asp Gln Met Val Phe Met Asp Tyr Arg Asp Tyr Thr	
280 285 290	
aac gag aaa gtt cgg agc tta gaa gct gag tat cca acg ttt ctg tac	1027
Asn Glu Lys Val Arg Ser Leu Glu Ala Glu Tyr Pro Thr Phe Leu Tyr	
295 300 305	
gcc atg cct atg aca aag tca aga ctc ttc ttc gag gag aca tgt ttg	1075
Ala Met Pro Met Thr Lys Ser Arg Leu Phe Phe Glu Glu Thr Cys Leu	
310 315 320	
gcc tca aaa gat gtc atg ccc ttt gat ttg cta aaa acg aag ctc atg	1123
Ala Ser Lys Asp Val Met Pro Phe Asp Leu Leu Lys Thr Lys Leu Met	
325 330 335 340	

tta aga tta gat aca ctc gga att cga att cta aag act tac gaa gag	1171
Leu Arg Leu Asp Thr Leu Gly Ile Arg Ile Leu Lys Thr Tyr Glu Glu	
345 350 355	
gag tgg tcc tat atc cca gtt ggt ggt tcc ttg cca aac acc gaa caa	1219
Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln	
360 365 370	
aag aat ctc gcc ttt ggt gct gcc gct agc atg gta cat ccc gca aca	1267
Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr	
375 380 385	
ggc tat tca gtt gtg aga tct ttg tct gaa gct cca aaa tat gca tca	1315
Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Tyr Ala Ser	
390 395 400	
gtc atc gca gag ata cta aga gaa gag act acc aaa cag atc aac agt	1363
Val Ile Ala Glu Ile Leu Arg Glu Glu Thr Thr Lys Gln Ile Asn Ser	
405 410 415 420	
aat att tca aga caa gct tgg gat act tta tgg cca cca gaa agg aaa	1411
Asn Ile Ser Arg Gln Ala Trp Asp Thr Leu Trp Pro Pro Glu Arg Lys	
425 430 435	
aga cag aga gca ttc ttt ctc ttt ggt ctt gca ctc ata gtt caa ttc	1459
Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu Ile Val Gln Phe	
440 445 450	
gat acc gaa ggc att aga agc ttc ttc cgt act ttc ttc cgc ctt cca	1507
Asp Thr Glu Gly Ile Arg Ser Phe Phe Arg Thr Phe Phe Arg Leu Pro	
455 460 465	
aaa tgg atg tgg caa ggg ttt cta gga tca aca tta aca tca gga gat	1555
Lys Trp Met Trp Gln Gly Phe Leu Gly Ser Thr Leu Thr Ser Gly Asp	
470 475 480	
ctc gtt ctc ttt gct tta tac atg ttc gtc att tca cca aac aat ttg	1603
Leu Val Leu Phe Ala Leu Tyr Met Phe Val Ile Ser Pro Asn Asn Leu	
485 490 495 500	
aga aaa ggt ctc atc aat cat ctc atc tct gat cca acc gga gca acc	1651
Arg Lys Gly Leu Ile Asn His Leu Ile Ser Asp Pro Thr Gly Ala Thr	
505 510 515	
atg ata aaa acc tat ctc aaa gta tgatttactt atcaactott aggttttgtt	1705
Met Ile Lys Thr Tyr Leu Lys Val	
520	
atatatatgt tgatttatct gaataatcga tcaaagaatg gatatgtgggt tactaggaag	1765
ttggaaacaa acatgtatag aatctaagga gtgacgaaa tggagatgga aac	1818
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20 25 30	
Ser Tyr Arg Asn Ile Arg Phe Gly Leu Cys Ser Val Arg Ala Ser Gly	
35 40 45	

Gly	Gly	Ser	Ser	Gly	Ser	Glu	Ser	Cys	Val	Ala	Val	Arg	Glu	Asp	Phe	50	55	60
Ala	Asp	Glu	Glu	Asp	Phe	Val	Lys	Ala	Gly	Gly	Ser	Glu	Ile	Leu	Phe	65	70	75
Val	Gln	Met	Gln	Gln	Asn	Lys	Asp	Met	Asp	Glu	Gln	Ser	Lys	Leu	Val	85	90	95
Asp	Lys	Leu	Pro	Pro	Ile	Ser	Ile	Gly	Asp	Gly	Ala	Leu	Asp	Leu	Val	100	105	110
Val	Ile	Gly	Cys	Gly	Pro	Ala	Gly	Leu	Ala	Leu	Ala	Ala	Glu	Ser	Ala	115	120	125
Lys	Leu	Gly	Leu	Lys	Val	Gly	Leu	Ile	Gly	Pro	Asp	Leu	Pro	Phe	Thr	130	135	140
Asn	Asn	Tyr	Gly	Val	Trp	Glu	Asp	Glu	Phe	Asn	Asp	Leu	Gly	Leu	Gln	145	150	155
Lys	Cys	Ile	Glu	His	Val	Trp	Arg	Glu	Thr	Ile	Val	Tyr	Leu	Asp	Asp	165	170	175
Asp	Lys	Pro	Ile	Thr	Ile	Gly	Arg	Ala	Tyr	Gly	Arg	Val	Ser	Arg	Arg	180	185	190
Leu	Leu	His	Glu	Glu	Leu	Leu	Arg	Cys	Val	Glu	Ser	Gly	Val	Ser		195	200	205
Tyr	Leu	Ser	Ser	Lys	Val	Asp	Ser	Ile	Thr	Glu	Ala	Ser	Asp	Gly	Leu	210	215	220
Arg	Leu	Val	Ala	Cys	Asp	Asp	Asn	Asn	Val	Ile	Pro	Cys	Arg	Leu	Ala	225	230	235
Thr	Val	Ala	Ser	Gly	Ala	Ala	Ser	Gly	Lys	Leu	Leu	Gln	Tyr	Glu	Val	245	250	255
Gly	Gly	Pro	Arg	Val	Cys	Val	Gln	Thr	Ala	Tyr	Gly	Val	Glu	Val	Glu	260	265	270
Val	Glu	Asn	Ser	Pro	Tyr	Asp	Pro	Asp	Gln	Met	Val	Phe	Met	Asp	Tyr	275	280	285
Arg	Asp	Tyr	Thr	Asn	Glu	Lys	Val	Arg	Ser	Leu	Glu	Ala	Glu	Tyr	Pro	290	295	300
Thr	Phe	Leu	Tyr	Ala	Met	Pro	Met	Thr	Lys	Ser	Arg	Leu	Phe	Phe	Glu	305	310	315
Glu	Thr	Cys	Leu	Ala	Ser	Lys	Asp	Val	Met	Pro	Phe	Asp	Leu	Leu	Lys	325	330	335
Thr	Lys	Leu	Met	Leu	Arg	Leu	Asp	Thr	Leu	Gly	Ile	Arg	Ile	Leu	Lys	340	345	350
Thr	Tyr	Glu	Glu	Glu	Trp	Ser	Tyr	Ile	Pro	Val	Gly	Gly	Ser	Leu	Pro	355	360	365
Asn	Thr	Glu	Gln	Lys	Asn	Leu	Ala	Phe	Gly	Ala	Ala	Ala	Ser	Met	Val	370	375	380
His	Pro	Ala	Thr	Gly	Tyr	Ser	Val	Val	Arg	Ser	Leu	Ser	Glu	Ala	Pro	385	390	395
Lys	Tyr	Ala	Ser	Val	Ile	Ala	Glu	Ile	Leu	Arg	Glu	Glu	Thr	Thr	Lys	405	410	415
Gln	Ile	Asn	Ser	Asn	Ile	Ser	Arg	Gln	Ala	Trp	Asp	Thr	Leu	Trp	Pro	420	425	430

Pro Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu
435 440 445
Ile Val Gln Phe Asp Thr Glu Gly Ile Arg Ser Phe Phe Arg Thr Phe
450 455 460
Phe Arg Leu Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser Thr Leu
465 470 475 480
Thr Ser Gly Asp Leu Val Leu Phe Ala Leu Tyr Met Phe Val Ile Ser
485 490 495
Pro Asn Asn Leu Arg Lys Gly Leu Ile Asn His Leu Ile Ser Asp Pro
500 505 510
Thr Gly Ala Thr Met Ile Lys Thr Tyr Leu Lys Val
515 520

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<212> DNA
<213> Oryza sativa
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<222> (1)..(1620)
<223> coding for epsilon-cyclase

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cgt gcg gcg tgg ggc gcc gcg gcg gcg ggt gcg ggg gcg gag gga agg 96
Arg Ala Ala Trp Gly Ala Ala Ala Ala Gly Ala Gly Ala Glu Gly Arg
20 25 30
agc agg agg gtt gtg ccg cgc gcg gtg gag ccg cgg cgg cgc ggg cgg 144
Ser Arg Arg Val Val Pro Arg Ala Val Glu Pro Arg Arg Arg Gly Arg
35 40 45
tgg atg gtg agg tgc gtg gcg acg gag aag cac aag gac gcg gcg gcg 192
Trp Met Val Arg Cys Val Ala Thr Glu Lys His Lys Asp Ala Ala Ala
50 55 60
cgg cgc ggc ggc gtg gag gtg gag ttc gcc gac gag gag gac tac gtc 240
Arg Arg Gly Gly Val Glu Val Glu Phe Ala Asp Glu Glu Asp Tyr Val
65 70 75 80
aag ggc ggc ggc ggc gag ctt ctc tac gtg caa atg cag gcg tcc aag 288
Lys Gly Gly Gly Gly Glu Leu Leu Tyr Val Gln Met Gln Ala Ser Lys
85 90 95
tcc atg gac agc cag tcc aag atc tcc tcc aag ctg ctg ccc ata ccc 336
Ser Met Asp Ser Gln Ser Lys Ile Ser Ser Lys Leu Leu Pro Ile Pro
100 105 110
gat gaa aat tca gtt ctt gat ttg gtc atc att ggc tgc ggt cca gct 384
Asp Glu Asn Ser Val Leu Asp Leu Val Ile Ile Gly Cys Gly Pro Ala
115 120 125
ggc tta tcc cta gca gca gag tca gct aag aaa ggg ctc aat gtt ggt 432
Gly Leu Ser Leu Ala Ala Glu Ser Ala Lys Lys Gly Leu Asn Val Gly
130 135 140

ctc att ggc cct gat ctt cca ttc acg aac aac tac ggt gtg tgg gag	480
Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu	
145 150 155 160	
gat gaa ttc aaa gac ctg ggc ctg gag agc tgc att gaa cat gtc tgg	528
Asp Glu Phe Lys Asp Leu Gly Leu Glu Ser Cys Ile Glu His Val Trp	
165 170 175	
aag gat act atc gtg tac cta gac ggt aac aag cca ata atg att ggc	576
Lys Asp Thr Ile Val Tyr Leu Asp Gly Asn Lys Pro Ile Met Ile Gly	
180 185 190	
cgt gcg tat ggc agg gtt cac agg gac ttg ctg cac gag gag ttg ctg	624
Arg Ala Tyr Gly Arg Val His Arg Asp Leu Leu His Glu Glu Leu Leu	
195 200 205	
aga cga tgc tat gac gct ggc gtc aca tac ctc agc tgc aag gtg gac	672
Arg Arg Cys Tyr Asp Ala Gly Val Thr Tyr Leu Ser Ser Lys Val Asp	
210 215 220	
aag atc atg gaa tct cct gat gga cat cgg gta gtc tgt tgt gaa ggg	720
Lys Ile Met Glu Ser Pro Asp Gly His Arg Val Val Cys Cys Glu Gly	
225 230 235 240	
gat cgt gag gta ctt tgc agg ctt gcc att gtt gca tct ggg gca gca	768
Asp Arg Glu Val Leu Cys Arg Leu Ala Ile Val Ala Ser Gly Ala Ala	
245 250 255	
tct ggt agg ctt cta gag tac gag gtt ggt ggt ccg cgt gtt tgt gtg	816
Ser Gly Arg Leu Leu Glu Tyr Glu Val Gly Gly Pro Arg Val Cys Val	
260 265 270	
cag act gca tat ggt gtc gaa gtc gag gtg gaa aac aat cca tat gat	864
Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Asn Pro Tyr Asp	
275 280 285	
ccc agc tta atg gtt ttc atg gac tac aga gat tgc ttc aaa gac aaa	912
Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Cys Phe Lys Asp Lys	
290 295 300	
ttc tca cat cct gag caa gga aat cca acg ttc ctc tat gcc atg ccc	960
Phe Ser His Pro Glu Gln Gly Asn Pro Thr Phe Leu Tyr Ala Met Pro	
305 310 315 320	
atg tca tcc aca cga att ttc ttt gag gaa aca tgc cta gct tct aaa	1008
Met Ser Ser Thr Arg Ile Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys	
325 330 335	
gaa gca atg ccc ttt gac ctc ctt aaa aag cgg ttg atg tct cgg ttg	1056
Glu Ala Met Pro Phe Asp Leu Leu Lys Lys Arg Leu Met Ser Arg Leu	
340 345 350	
gat gca atg gga gtt cat att cga aaa gta tac gag gag gaa tgg tcc	1104
Asp Ala Met Gly Val His Ile Arg Lys Val Tyr Glu Glu Glu Trp Ser	
355 360 365	
tac att cct gtt gga ggg tcc tta cca aat aca gac cag aaa aat ctc	1152
Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Asp Gln Lys Asn Leu	
370 375 380	
gca ttt ggt gcg gca gca agt atg gtg cat cct gca acc gga tac tcg	1200
Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser	
385 390 395 400	

gtg gtt aga tca ttg tct gaa gct cca aga tat gca tct gtg ata tct	1248
Val Val Arg Ser Leu Ser Glu Ala Pro Arg Tyr Ala Ser Val Ile Ser	
405 410 415	
gat atc ttg aga aac cgt gtc tac cct gga gaa tat ttg cct gga acc	1296
Asp Ile Leu Arg Asn Arg Val Tyr Pro Gly Glu Tyr Leu Pro Gly Thr	
420 425 430	
tct caa agt tcc agt cca tca atg ctt gca tgg aga aca tta tgg ccc	1344
Ser Gln Ser Ser Ser Pro Ser Met Leu Ala Trp Arg Thr Leu Trp Pro	
435 440 445	
caa gaa cgg aaa cgt caa cga tca ttc ttc ctt ttt ggg ctg gct ttg	1392
Gln Glu Arg Lys Arg Gln Arg Ser Phe Phe Leu Phe Gly Leu Ala Leu	
450 455 460	
ata atc caa ctg aat aac gaa ggc att cag aca ttc ttt gaa acc ttt	1440
Ile Ile Gln Leu Asn Asn Glu Gly Ile Gln Thr Phe Phe Glu Thr Phe	
465 470 475 480	
ttc cgg ttg ccc aaa tgg atg tgg cga gga ttc ctt ggt tcg acg ctt	1488
Phe Arg Leu Pro Lys Trp Met Trp Arg Gly Phe Leu Gly Ser Thr Leu	
485 490 495	
tct tca gtg gat ctc ata ctc ttt gca ttc tac atg ttc aca att gcg	1536
Ser Ser Val Asp Leu Ile Leu Phe Ala Phe Tyr Met Phe Thr Ile Ala	
500 505 510	
ccg aac caa atg cga atg aac ctt gtc aga cat ctc ctc tct gat ccg	1584
Pro Asn Gln Met Arg Met Asn Leu Val Arg His Leu Leu Ser Asp Pro	
515 520 525	
acc ggc tca acg atg atc aag acc tac ctg acc ttg taa	1623
Thr Gly Ser Thr Met Ile Lys Thr Tyr Leu Thr Leu	
530 535 540	
<210> 16	
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<212> PRT	
<213> Oryza sativa	
<400> 16	
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Arg Ala Ala Trp Gly Ala Ala Ala Ala Gly Ala Gly Ala Glu Gly Arg	
20 25 30	
Ser Arg Arg Val Val Pro Arg Ala Val Glu Pro Arg Arg Arg Gly Arg	
35 40 45	
Trp Met Val Arg Cys Val Ala Thr Glu Lys His Lys Asp Ala Ala Ala	
50 55 60	
Arg Arg Gly Gly Val Glu Val Glu Phe Ala Asp Glu Glu Asp Tyr Val	
65 70 75 80	
Lys Gly Gly Gly Gly Glu Leu Leu Tyr Val Gln Met Gln Ala Ser Lys	
85 90 95	
Ser Met Asp Ser Gln Ser Lys Ile Ser Ser Lys Leu Leu Pro Ile Pro	
100 105 110	
Asp Glu Asn Ser Val Leu Asp Leu Val Ile Ile Gly Cys Gly Pro Ala	
115 120 125	
Gly Leu Ser Leu Ala Ala Glu Ser Ala Lys Lys Gly Leu Asn Val Gly	
130 135 140	

Thr Gly Ser Thr Met Ile Lys Thr Tyr Leu Thr Leu
530 535 540

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<211> 10
<212> PRT
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motif for epsilon-cyclase
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<221> VARIANT
<222> (2)
<223> G/C variation
<220>
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<222> (8)
<223> A/S variation
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<221> VARIANT
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<223> V/L variation
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1 5 10

<210> 18
<211> 8
<212> PRT
<213> Artificial sequence
<220>
<223> Description of the artificial sequence: protein
motif for epsilon-cyclase
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<222> (1)
<223> L/I variation
<220>
<221> VARIANT
<222> (2)
<223> N/G/S variation
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<221> VARIANT
<222> (7)
<223> K/R variation
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<221> VARIANT
<222> (8)
<223> V/L variation
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1 5

<210> 19
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motif for epsilon-cyclase
<220>
<221> VARIANT
<222> (6)
<223> A/V variation
<220>
<221> VARIANT
<222> (8)
<223> P/A variation
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Pro Thr Phe Leu Tyr Ala Met Pro
1 5
<210> 21
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<212> PRT
<213> Artificial sequence
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<223> Description of the artificial sequence: protein
motif for epsilon-cyclase
<220>
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<223> S/A-variation
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<221> VARIANT
<222> (11)
<223> M/S variation
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<221> VARIANT
<222> (13)
<223> A/V variation

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<210> 22
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<212> PRT
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<220>
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      motif for epsilon-cyclase

<220>
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<222> (7)
<223> R/K variation

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<210> 23
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<212> DNA
<213> Lactuca sativa

<220>
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<222> (77)..(1675)
<223> coding for epsilon-cyclase

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tgtgatagaa gaatca atg gag tgc ttt gga gct cga aac atg acg gca aca 112
      Met Glu Cys Phe Gly Ala Arg Asn Met Thr Ala Thr
      1             5             10

atg gcg gtt ttt acg tgc cct aga ttc acg gac tgt aat atc agg cac 160
Met Ala Val Phe Thr Cys Pro Arg Phe Thr Asp Cys Asn Ile Arg His
      15             20             25

aaa ttt tcg tta ctg aaa caa cga aga ttt act aat tta tca gca tcg 208
Lys Phe Ser Leu Leu Lys Gln Arg Arg Phe Thr Asn Leu Ser Ala Ser
      30             35             40

tct tcg ttg cgt caa att aag tgc agc gct aaa agc gac cgt tgt gta 256
Ser Ser Leu Arg Gln Ile Lys Cys Ser Ala Lys Ser Asp Arg Cys Val
      45             50             55             60

gtg gat aaa caa ggg att tcc gta gca gac gaa gaa gat tat gtg aag 304
Val Asp Lys Gln Gly Ile Ser Val Ala Asp Glu Glu Asp Tyr Val Lys
      65             70             75

gcc ggt gga tcg gag ctg ttt ttt gtt caa atg cag cgg act aag tcc 352
Ala Gly Gly Ser Glu Leu Phe Phe Val Gln Met Gln Arg Thr Lys Ser
      80             85             90

atg gaa agc cag tct aaa ctt tcc gaa aag cta gca cag ata cca att 400
Met Glu Ser Gln Ser Lys Leu Ser Glu Lys Leu Ala Gln Ile Pro Ile
      95             100             105

gga aat tgc ata ctt gat ctg gtt gta atc ggt tgt ggc cct gct ggc 448
Gly Asn Cys Ile Leu Asp Leu Val Val Ile Gly Cys Gly Pro Ala Gly
      110             115             120

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ctt gct ctt gct gca gag tca gcc aaa cta ggg ttg aac gtt gga ctc	496
Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Gly Leu	
125 130 135 140	
att ggc cct gat ctt cct ttt aca aac aat tat ggt gtt tgg cag gat	544
Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Gln Asp	
145 150 155	
gaa ttt ata ggt ctt gga ctt gaa gga tgc att gaa cat tct tgg aaa	592
Glu Phe Ile Gly Leu Gly Leu Glu Gly Cys Ile Glu His Ser Trp Lys	
160 165 170	
gat act ctt gta tac ctt gat gat gct gat ccc atc cgc ata ggt cgt	640
Asp Thr Leu Val Tyr Leu Asp Asp Ala Asp Pro Ile Arg Ile Gly Arg	
175 180 185	
gca tat ggc aga gtt cat cgt gat tta ctt cat gaa gag ttg tta aga	688
Ala Tyr Gly Arg Val His Arg Asp Leu Leu His Glu Glu Leu Leu Arg	
190 195 200	
agg tgt gtg gaa tca ggt gtt tca tat cta agc tcc aaa gta gaa aga	736
Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Arg	
205 210 215 220	
atc act gaa gct cca aat ggc tat agt ctc att gaa tgt gaa ggc aat	784
Ile Thr Glu Ala Pro Asn Gly Tyr Ser Leu Ile Glu Cys Glu Gly Asn	
225 230 235	
atc acc att cca tgc agg ctt gct act gtt gca tca ggg gca gct tca	832
Ile Thr Ile Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser	
240 245 250	
ggg aaa ttt ctg gag tat gaa ctt ggg ggt ccc cgt gtt tgt gtc caa	880
Gly Lys Phe Leu Glu Tyr Glu Leu Gly Gly Pro Arg Val Cys Val Gln	
255 260 265	
aca gct tat ggt ata gag gtt gag gtt gaa aac aac ccc tat gat cca	928
Thr Ala Tyr Gly Ile Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro	
270 275 280	
gat cta atg gtg ttc atg gat tat aga gac ttc tca aaa cat aaa ccg	976
Asp Leu Met Val Phe Met Asp Tyr Arg Asp Phe Ser Lys His Lys Pro	
285 290 295 300	
gaa tct tta gaa gca aaa tat ccg act ttc ctc tat gtc atg gcc atg	1024
Glu Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu Tyr Val Met Ala Met	
305 310 315	
tct cca aca aaa ata ttc ttc gag gaa act tgt tta gct tca aga gaa	1072
Ser Pro Thr Lys Ile Phe Phe Glu Glu Thr Cys Leu Ala Ser Arg Glu	
320 325 330	
gcc atg cct ttc aat ctt cta aag tcc aaa ctc atg tca cga tta aag	1120
Ala Met Pro Phe Asn Leu Leu Lys Ser Lys Leu Met Ser Arg Leu Lys	
335 340 345	
gca atg ggt atc cga ata aca aga acg tac gaa gag gaa tgg tcg tat	1168
Ala Met Gly Ile Arg Ile Thr Arg Thr Tyr Glu Glu Glu Trp Ser Tyr	
350 355 360	
atc ccc gta ggt gga tcg tta cct aat aca gaa caa aag aat ctc gca	1216
Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys Asn Leu Ala	
365 370 375 380	

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ttt ggt gct gca gct agt atg gtg cac cct gcc aca ggg tat tca gtt 1264
Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val
385 390 395

ggt cga tct ttg tca gaa gct cct aat tat gca gca gtc att gct aag 1312
Val Arg Ser Leu Ser Glu Ala Pro Asn Tyr Ala Ala Val Ile Ala Lys
400 405 410

att tta aga caa gat caa tct aaa gag atg att tct ctt gga aaa tac 1360
Ile Leu Arg Gln Asp Gln Ser Lys Glu Met Ile Ser Leu Gly Lys Tyr
415 420 425

act aac att tca aaa caa gca tgg gaa aca ttg tgg cca ctt gaa agg 1408
Thr Asn Ile Ser Lys Gln Ala Trp Glu Thr Leu Trp Pro Leu Glu Arg
430 435 440

aaa aga cag cga gcc ttc ttt cta ttc gga cta tca cac atc gtg cta 1456
Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ser His Ile Val Leu
445 450 455 460

atg gat cta gag gga aca cgt aca ttt ttc cgt act ttc ttt cgt ttg 1504
Met Asp Leu Glu Gly Thr Arg Thr Phe Phe Arg Thr Phe Phe Arg Leu
465 470 475

ccc aaa tgg atg tgg tgg gga ttt ttg ggg tct tct tta tct tca acg 1552
Pro Lys Trp Met Trp Trp Gly Phe Leu Gly Ser Ser Leu Ser Ser Thr
480 485 490

gat ttg ata ata ttt gcg ctt tat atg ttt gtg ata gca cct cac agc 1600
Asp Leu Ile Ile Phe Ala Leu Tyr Met Phe Val Ile Ala Pro His Ser
495 500 505

ttg aga atg gaa ctg gtt aga cat cta ctt tct gat ccg aca ggg gca 1648
Leu Arg Met Glu Leu Val Arg His Leu Leu Ser Asp Pro Thr Gly Ala
510 515 520

act atg gta aaa gca tat ctc act ata tagatttaga ttatataaat 1695
Thr Met Val Lys Ala Tyr Leu Thr Ile
525 530

aatacccata tcttgcatat atataagcct tattttatttc ttttgtatcc ttacaacaac 1755
atactcgtaa attatatggt tttta 1780

<210> 24
<211> 533
<212> PRT
<213> Lactuca sativa

<400> 24
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20 25 30
Leu Lys Gln Arg Arg Phe Thr Asn Leu Ser Ala Ser Ser Ser Leu Arg
35 40 45
Gln Ile Lys Cys Ser Ala Lys Ser Asp Arg Cys Val Val Asp Lys Gln
50 55 60
Gly Ile Ser Val Ala Asp Glu Glu Asp Tyr Val Lys Ala Gly Gly Ser
65 70 75 80
Glu Leu Phe Phe Val Gln Met Gln Arg Thr Lys Ser Met Glu Ser Gln
85 90 95

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Ser Lys Leu Ser Glu Lys Leu Ala Gln Ile Pro Ile Gly Asn Cys Ile
100 105 110

Leu Asp Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala
115 120 125

Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Gly Leu Ile Gly Pro Asp
130 135 140

Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Gln Asp Glu Phe Ile Gly
145 150 155 160

Leu Gly Leu Glu Gly Cys Ile Glu His Ser Trp Lys Asp Thr Leu Val
165 170 175

Tyr Leu Asp Asp Ala Asp Pro Ile Arg Ile Gly Arg Ala Tyr Gly Arg
180 185 190

Val His Arg Asp Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu
195 200 205

Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala
210 215 220

Pro Asn Gly Tyr Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro
225 230 235 240

Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Phe Leu
245 250 255

Glu Tyr Glu Leu Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly
260 265 270

Ile Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro Asp Leu Met Val
275 280 285

Phe Met Asp Tyr Arg Asp Phe Ser Lys His Lys Pro Glu Ser Leu Glu
290 295 300

Ala Lys Tyr Pro Thr Phe Leu Tyr Val Met Ala Met Ser Pro Thr Lys
305 310 315 320

Ile Phe Phe Glu Glu Thr Cys Leu Ala Ser Arg Glu Ala Met Pro Phe
325 330 335

Asn Leu Leu Lys Ser Lys Leu Met Ser Arg Leu Lys Ala Met Gly Ile
340 345 350

Arg Ile Thr Arg Thr Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly
355 360 365

Gly Ser Leu Pro Asn Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala
370 375 380

Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu
385 390 395 400

Ser Glu Ala Pro Asn Tyr Ala Ala Val Ile Ala Lys Ile Leu Arg Gln
405 410 415

Asp Gln Ser Lys Glu Met Ile Ser Leu Gly Lys Tyr Thr Asn Ile Ser
420 425 430

Lys Gln Ala Trp Glu Thr Leu Trp Pro Leu Glu Arg Lys Arg Gln Arg
435 440 445

Ala Phe Phe Leu Phe Gly Leu Ser His Ile Val Leu Met Asp Leu Glu
450 455 460

Gly Thr Arg Thr Phe Phe Arg Thr Phe Phe Arg Leu Pro Lys Trp Met
465 470 475 480

Trp Trp Gly Phe Leu Gly Ser Ser Leu Ser Ser Thr Asp Leu Ile Ile
485 490 495
Phe Ala Leu Tyr Met Phe Val Ile Ala Pro His Ser Leu Arg Met Glu
500 505 510
Leu Val Arg His Leu Leu Ser Asp Pro Thr Gly Ala Thr Met Val Lys
515 520 525
Ala Tyr Leu Thr Ile
530

<210> 25
<211> 1848
<212> DNA
<213> Adonis palaestina
<220>
<221> CDS
<222> (116)..(1702)
<223> coding for epsilon cyclase
<400> 25

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ccattttctt gttttctctt caaaacaaca aactaatgtg acggagtatc tagct atg 118
Met
1
gaa cta ctt ggt gtt cgc aac ctg atc tct tct tgc cct gtc tgg act 166
Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp Thr
5 10 15
ttt gga aca aga aac ctt agt agt tca aaa cta gct tat aac ata cat 214
Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile His
20 25 30
cga tat ggt tct tct tgt aga gta gat ttt caa gtg agg gct gat ggt 262
Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp Gly
35 40 45
gga agc ggg agt aga act tct gtt gct tat aaa gag ggt ttt gtg gac 310
Gly Ser Gly Ser Arg Thr Ser Val Ala Tyr Lys Glu Gly Phe Val Asp
50 55 60 65
gag gag gat ttt atc aaa gct ggt ggt tct gag ctt ttg ttt gtc caa 358
Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val Gln
70 75 80
atg cag caa aca aag tct atg gag aaa cag gcc aag ctg gcc gat aag 406
Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp Lys
85 90 95
ttg cca cca ata cct ttc gga gaa tct gtg atg gac ttg gtt gta ata 454
Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val Ile
100 105 110
ggt tgt gga cct gct ggt ctt tca ctg gct gca gaa gct gct aag cta 502
Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys Leu
115 120 125
ggc ttg aaa gtt ggc ctt att ggt cct gat ctt cct ttt aca aat aat 550
Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn
130 135 140 145

tat ggt gtg tgg gaa gac gag ttc aaa gat ctt gga ctt gaa cgt tgt	598
Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg Cys	
150 155 160	
atc gag cat gct tgg aag gac acc atc gta tat ctt gac aat gat gct	646
Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp Ala	
165 170 175	
cct gtc ctt att ggt cgt gca tat gga cga gtt agc cgg cat ttg ctg	694
Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu	
180 185 190	
cat gaa gag ttg ctg aaa agg tgt gtc gag tca ggt gta tca tat ctg	742
His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr Leu	
195 200 205	
aat tct aaa gtg gaa agg atc act gaa gct ggt gat ggc cat agt ctt	790
Asn Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser Leu	
210 215 220 225	
gta gtt tgt gaa aac gac atc ttt atc cct tgc agg ctt gct act gtt	838
Val Val Cys Glu Asn Asp Ile Phe Ile Pro Cys Arg Leu Ala Thr Val	
230 235 240	
gca tct gga gca gct tca ggg aaa ctt ttg gag tat gaa gta ggt ggc	886
Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly Gly	
245 250 255	
cct cgt gtt tgt gtc caa act gct tat ggt gtg gag gtt gag gtg gag	934
Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu	
260 265 270	
aac aat cca tac gat ccc aac tta atg gta ttt atg gac tac aga gac	982
Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg Asp	
275 280 285	
tat atg caa cag aaa tta cag tgc tcg gaa gaa gaa tat cca aca ttt	1030
Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr Phe	
290 295 300 305	
ctc tat gtc atg ccc atg tcg cca aca aga ctt ttt ttt gag gaa acc	1078
Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu Thr	
310 315 320	
tgt ttg gcc tca aaa gat gcc atg cct ttc gat cta ctg aag aga aaa	1126
Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg Lys	
325 330 335	
cta atg tca cga ttg aag act ctg ggt atc caa gtt aca aaa att tat	1174
Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Ile Tyr	
340 345 350	
gaa gag gaa tgg tct tat att cct gtt ggg ggt tct tta cca aac aca	1222
Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr	
355 360 365	
gag caa aag aac cta gca ttt ggt gct gca gca agc atg gtg cat cca	1270
Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro	
370 375 380 385	
gca aca ggc tat tcg gtt gta cga tca cta tca gaa gct cca aaa tat	1318
Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Tyr	
390 395 400	

gct tct gta att gca aag att ttg aag caa gat aac tct gca tat gtg 1366
 Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr Val
 405 410 415
 gtt tct gga caa agc agt gca gta aac att tca atg caa gca tgg agc 1414
 Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp Ser
 420 425 430
 agt ctt tgg cca aag gag cga aaa cgt caa aga gca ttc ttt ctt ttc 1462
 Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe
 435 440 445
 ggg tta gag ctt att gtg cag cta gat att gaa gca acc aga acg ttc 1510
 Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr Phe
 450 455 460 465
 ttt aga acc ttc ttc cgc ttg cca act tgg atg tgg tgg ggt ttc ctt 1558
 Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe Leu
 470 475 480
 ggg tct tca cta tca tct ttc gat ctt gta ttg ttt tcc atg tac atg 1606
 Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr Met
 485 490 495
 ttt gtt ttg gcc ccg aac agc atg agg atg tca ctt gtg aga cat ttg 1654
 Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His Leu
 500 505 510
 ctt tca gat cct tct ggt gca gtt atg gtt aaa gct tac ctc gaa agg 1702
 Leu Ser Asp Pro Ser Gly Ala Val Met Val Lys Ala Tyr Leu Glu Arg
 515 520 525
 taatctgttt tatgaaacta tagtgtctca ttaaataaat gaggatcctt cgtatatgta 1762
 tatgatcatc tctatgtata tcctatatcc taatctcata aagtaatcga aaattcattg 1822
 atagaaaaaaaa aaaaaaaaaa aaaaaa 1848
 <210> 26
 <211> 529
 <212> PRT
 <213> Adonis palaestina
 <400> 26
 Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp
 1 5 10 15
 Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile
 20 25 30
 His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp
 35 40 45
 Gly Gly Ser Gly Ser Arg Thr Ser Val Ala Tyr Lys Glu Gly Phe Val
 50 55 60
 Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val
 65 70 75 80
 Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp
 85 90 95
 Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val
 100 105 110
 Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys
 115 120 125

Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn			
130	135	140	
Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg			
145	150	155	160
Cys Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp			
	165	170	175
Ala Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu			
	180	185	190
Leu His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr			
	195	200	205
Leu Asn Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser			
	210	215	220
Leu Val Val Cys Glu Asn Asp Ile Phe Ile Pro Cys Arg Leu Ala Thr			
225	230	235	240
Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly			
	245	250	255
Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val			
	260	265	270
Glu Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg			
	275	280	285
Asp Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr			
	290	295	300
Phe Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu			
305	310	315	320
Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg			
	325	330	335
Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Ile			
	340	345	350
Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn			
	355	360	365
Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His			
	370	375	380
Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys			
385	390	395	400
Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr			
	405	410	415
Val Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp			
	420	425	430
Ser Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu			
	435	440	445
Phe Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr			
	450	455	460
Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe			
465	470	475	480
Leu Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr			
	485	490	495
Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His			
	500	505	510

Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Lys Ala Tyr Leu Glu
 515 520 525

Arg

<210> 27

<211> 1898

<212> DNA

<213> Adonis palaestina

<220>

<221> CDS

<222> (113)..(1699)

<223> coding for epsilon-cyclase

<400> 27

aaaggagtgt tctattaatg ttactgtgcg attcttgcaa caattatatt caaactccat 60
 tttcttcttt tctcttcaaa acaacaaact aatgtgagca gagtatctgg ct atg gaa 118
 Met Glu
 1

cta ctt ggt gtt cgc aac ctc atc tct tct tgc cct gtg tgg act ttt 166
 Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp Thr Phe
 5 10 15

gga aca aga aac ctt agt agt tca aaa cta gct tat aac ata cat cga 214
 Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile His Arg
 20 25 30

tat ggt tct tct tgt aga gta gat ttt caa gtg aga gct gat ggt gga 262
 Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp Gly Gly
 35 40 45 50

agc ggg agt aga agt tct gtt gct tat aaa gag ggt ttt gtg gat gaa 310
 Ser Gly Ser Arg Ser Ser Val Ala Tyr Lys Glu Gly Phe Val Asp Glu
 55 60 65

gag gat ttt atc aaa gct ggt ggt tct gag ctt ttg ttt gtc caa atg 358
 Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val Gln Met
 70 75 80

cag caa aca aag tct atg gag aaa cag gcc aag ctc gcc gat aag ttg 406
 Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp Lys Leu
 85 90 95

cca cca ata cct ttt gga gaa tcc gtg atg gac ttg gtt gta ata ggt 454
 Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val Ile Gly
 100 105 110

tgt gga cct gct ggt ctt tca ctg gct gca gaa gct gct aag cta ggg 502
 Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys Leu Gly
 115 120 125 130

ttg aaa gtt ggc ctt att ggt cct gat ctt cct ttt aca aat aat tat 550
 Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr
 135 140 145

ggt gtg tgg gaa gac gag ttc aaa gat ctt gga ctt gaa cgt tgt atc 598
 Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg Cys Ile
 150 155 160

gag cat gct tgg aag gac acc atc gta tat ctt gat aat gat gct cct Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp Ala Pro 165 170 175	646
gtc ctt att ggt cgt gca tat gga cga gtt agt cga cat ttg cta cat Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu His 180 185 190	694
gag gag ttg ctg aaa agg tgt gtg gag tca ggt gta tca tat ctg gat Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Asp 195 200 205 210	742
tct aaa gtg gaa agg atc act gaa gct ggt gat ggc cat agc ctt gta Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser Leu Val 215 220 225	790
gtt tgt gaa aat gag atc ttt atc cct tgc agg ctt gct act gtt gca Val Cys Glu Asn Glu Ile Phe Ile Pro Cys Arg Leu Ala Thr Val Ala 230 235 240	838
tct gga gca gct tca ggg aaa ctt ttg gag tat gaa gta ggt ggc cct Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly Gly Pro 245 250 255	886
cgt gtt tgt gtc caa acc gct tat ggg gtg gag gtt gag gtg gag aac Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn 260 265 270	934
aat cca tac gat ccc aac tta atg gta ttc atg gac tac aga gac tat Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg Asp Tyr 275 280 285 290	982
atg caa cag aaa tta cag tgc tcg gaa gaa gaa tat cca aca ttt ctc Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Tyr Pro Thr Phe Leu 295 300 305	1030
tat gtc atg ccc atg tcg cca aca aga ctt ttt ttt gag gaa acc tgt Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu Thr Cys 310 315 320	1078
ttg gcc tca aaa gat gcc atg cca ttc gat cta ctg aag aga aaa ctg Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg Lys Leu 325 330 335	1126
atg tca cga ttg aag act ctg ggt atc caa gtt aca aaa gtt tat gaa Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Val Tyr Glu 340 345 350	1174
gag gaa tgg tca tat att cct gtt ggt ggt tct tta cca aac aca gag Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu 355 360 365 370	1222
caa aag aac cta gca ttt ggt gct gca gca agc atg gtg cat cca gca Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala 375 380 385	1270
aca ggc tat tcg gtt gta cgg tca ctg tca gaa gct cca aaa tat gct Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Tyr Ala 390 395 400	1318
tct gta att gca aag att ttg aag caa gat aac tct gcg tat gtg gtt Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr Val Val 405 410 415	1366

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tct gga caa agt agt gca gta aac att tca atg caa gca tgg agc agt 1414
Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp Ser Ser
420 425 430

ctt tgg cca aag gag cga aaa cgt caa aga gca ttc ttt ctt ttt gga 1462
Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly
435 440 445 450

tta gag ctt att gtg cag cta gat att gaa gca acc aga aca ttc ttt 1510
Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr Phe Phe
455 460 465

aga acc ttc ttc cgc ttg cca act tgg atg tgg tgg ggt ttc ctt ggg 1558
Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe Leu Gly
470 475 480

tct tca cta tca tct ttc gat ctc gtc ttg ttt tcc atg tac atg ttt 1606
Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr Met Phe
485 490 495

gtt ttg gcg cca aac agc atg agg atg tca ctt gtg aga cat ttg ctt 1654
Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His Leu Leu
500 505 510

tca gat cct tct ggt gca gtt atg gta aga gct tac ctc gaa agg 1699
Ser Asp Pro Ser Gly Ala Val Met Val Arg Ala Tyr Leu Glu Arg
515 520 525

tagtctcattc tattattaaa ctctagtggt tcaccaaata aatgaggatc cttcgaaatgt 1759
gtatatgatac atctctatgt atatcctgta ctctaattctc ataaagtaaa tgccggggttt 1819
gatattgttg tgtcaaaccg gccaatgata taaagtaaatt ttattgatac aaaagtagtt 1879
tttttcctta aaaaaaaaaa 1898

<210> 28
<211> 529
<212> PRT
<213> Adonis palaestina
<400> 28
Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp
1 5 10 15
Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile
20 25 30
His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp
35 40 45
Gly Gly Ser Gly Ser Arg Ser Ser Val Ala Tyr Lys Glu Gly Phe Val
50 55 60
Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val
65 70 75 80
Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp
85 90 95
Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val
100 105 110
Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys
115 120 125
Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn
130 135 140

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Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg
145 150 155 160
Cys Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp
165 170 175
Ala Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu
180 185 190
Leu His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr
195 200 205
Leu Asp Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser
210 215 220
Leu Val Val Cys Glu Asn Glu Ile Phe Ile Pro Cys Arg Leu Ala Thr
225 230 235 240
Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly
245 250 255
Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val
260 265 270
Glu Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg
275 280 285
Asp Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr
290 295 300
Phe Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu
305 310 315 320
Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg
325 330 335
Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Val
340 345 350
Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn
355 360 365
Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His
370 375 380
Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys
385 390 395 400
Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr
405 410 415
Val Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp
420 425 430
Ser Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu
435 440 445
Phe Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr
450 455 460
Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe
465 470 475 480
Leu Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr
485 490 495
Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His
500 505 510
Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Arg Ala Tyr Leu Glu
515 520 525

Arg

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<210> 29
<211> 1661
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cag ttt cat ggg ttt gag aga tta tgc agt aac aat cca tac cct tca 97
Gln Phe His Gly Phe Glu Arg Leu Cys Ser Asn Asn Pro Tyr Pro Ser
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agg gtt agg ctt ggt gtg aag aaa agg gct atc aaa att gtc tct agt 145
Arg Val Arg Leu Gly Val Lys Lys Arg Ala Ile Lys Ile Val Ser Ser
      35              40              45
gta gtg agt ggt agc gct gct ctt ttg gat ctt gtt cct gaa act aag 193
Val Val Ser Gly Ser Ala Ala Leu Leu Asp Leu Val Pro Glu Thr Lys
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aag gag aat ctt gac ttt gag ctt cct ttg tac gac act tcc aag agt 241
Lys Glu Asn Leu Asp Phe Glu Leu Pro Leu Tyr Asp Thr Ser Lys Ser
      65              70              75              80
caa gtt gtt gat ttg gct att gtt ggt ggt ggt cct gct ggt tta gcc 289
Gln Val Val Asp Leu Ala Ile Val Gly Gly Gly Pro Ala Gly Leu Ala
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gtg gct cag cag gtt tct gaa gct gga ctc tct gtt tgt tcc att gat 337
Val Ala Gln Gln Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile Asp
      100             105             110
cct tct cct aag ctc ata tgg cct aac aat tat gga gtt tgg gtt gat 385
Pro Ser Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val Asp
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gag ttt gag gct atg gat tta cta gac tgc ctg gat acc aca tgg tct 433
Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Thr Thr Trp Ser
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Gly Ala Val Val Tyr Val Asp Glu Gly Val Lys Lys Asp Leu Ser Arg
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cct tat ggg aga gtt aac cgg aaa cag ctc aaa tcc aaa atg ctt cag 529
Pro Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys Met Leu Gln
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aaa tgt att acc aac ggt gtt aaa ttt cat cag tct aag gtc act aat 577
Lys Cys Ile Thr Asn Gly Val Lys Phe His Gln Ser Lys Val Thr Asn
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 Phe Gly Leu Ser Leu Phe Ser His Ala Ser Asn Thr Ser Arg Leu Glu
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 Lys Glu Asn Leu Asp Phe Glu Leu Pro Leu Tyr Asp Thr Ser Lys Ser
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 85 90 95
 Val Ala Gln Gln Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile Asp
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 Pro Ser Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val Asp
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 Pro Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys Met Leu Gln
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 Lys Cys Ile Thr Asn Gly Val Lys Phe His Gln Ser Lys Val Thr Asn
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 Lys Ile Gln Ala Ser Val Val Leu Asp Ala Thr Gly Phe Ser Arg Cys
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Gly Ile Ile Ala Glu Val Asp Gly His Pro Phe Asp Val Asp Lys Met
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 Val Phe Met Asp Trp Arg Asp Lys His Leu Asp Ser Tyr Pro Glu Leu
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 325 330 335
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 Ala Thr Arg Arg Phe Phe Asp Ala Phe Phe Asp Leu Gln Pro His Tyr
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Asn Tyr Gly Val Trp Glu Asp Glu Phe Arg Asp Leu Gly Leu Glu Gly	
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Cys Ile Glu His Val Trp Arg Asp Thr Val Val Tyr Ile Asp Glu Asp	
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Leu Ser Ser Lys Val Glu Ser Ile Thr Glu Ser Thr Ser Gly His Arg	
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135 140 145	
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Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly	
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Phe Leu Tyr Val Met Pro Met Ser Ser Thr Arg Val Phe Phe Glu Glu	
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act tgt ttg gca tcg aaa gat ggt tta cgt ttt gac ata ttg aag aaa	776
Thr Cys Leu Ala Ser Lys Asp Gly Leu Arg Phe Asp Ile Leu Lys Lys	
230 235 240	
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Lys Leu Met Ala Arg Leu Glu Arg Leu Gly Ile Gln Val Leu Lys Thr	
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tat gaa gag gaa tgg tca tat att cca gtt ggt ggt tcc tta cca aat 872
Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn
265 270 275

aca gaa caa aga aac ctc gca ttt ggt gct gct gct agc atg gtg cat 920
Thr Glu Gln Arg Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His
280 285 290

cca gcc act ggc tac tca gta gtc aga tca ctg tca gag gct cca aac 968
Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Asn
295 300 305

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Tyr Ala Ser Ala Ile Ala Tyr Ile Leu Lys His Asp His Ser Arg Gly
310 315 320

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325 330 335 340

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360 365 370

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425 430 435

tta tagttagttt gtattttcca tatttcagcc cttgtttggg atatttttggg 1405
Leu

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35 40 45

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Gly	Leu	Glu	Gly	Cys	Ile	Glu	His	Val	Trp	Arg	Asp	Thr	Val	Val	Tyr	65	70	75
Ile	Asp	Glu	Asp	Glu	Pro	Ile	Leu	Ile	Gly	Arg	Ala	Tyr	Gly	Arg	Val	85	90	95
Ser	Arg	His	Leu	Leu	His	Glu	Glu	Leu	Leu	Arg	Arg	Cys	Val	Glu	Ser	100	105	110
Gly	Val	Ser	Tyr	Leu	Ser	Ser	Lys	Val	Glu	Ser	Ile	Thr	Glu	Ser	Thr	115	120	125
Ser	Gly	His	Arg	Leu	Val	Ala	Cys	Glu	His	Asp	Met	Ile	Val	Pro	Cys	130	135	140
Arg	Leu	Ala	Thr	Val	Ala	Ser	Gly	Ala	Ala	Ser	Gly	Lys	Leu	Leu	Glu	145	150	155
Tyr	Glu	Val	Gly	Gly	Pro	Lys	Val	Ser	Val	Gln	Thr	Ala	Tyr	Gly	Val	165	170	175
Glu	Val	Glu	Val	Glu	Asn	Asn	Pro	Tyr	Asp	Pro	Ser	Leu	Met	Val	Phe	180	185	190
Met	Asp	Tyr	Arg	Asp	Cys	Thr	Lys	Gln	Glu	Val	Pro	Ser	Phe	Glu	Ser	195	200	205
Asp	Asn	Pro	Thr	Phe	Leu	Tyr	Val	Met	Pro	Met	Ser	Ser	Thr	Arg	Val	210	215	220
Phe	Phe	Glu	Glu	Thr	Cys	Leu	Ala	Ser	Lys	Asp	Gly	Leu	Arg	Phe	Asp	225	230	235
Ile	Leu	Lys	Lys	Lys	Leu	Met	Ala	Arg	Leu	Glu	Arg	Leu	Gly	Ile	Gln	245	250	255
Val	Leu	Lys	Thr	Tyr	Glu	Glu	Glu	Trp	Ser	Tyr	Ile	Pro	Val	Gly	Gly	260	265	270
Ser	Leu	Pro	Asn	Thr	Glu	Gln	Arg	Asn	Leu	Ala	Phe	Gly	Ala	Ala	Ala	275	280	285
Ser	Met	Val	His	Pro	Ala	Thr	Gly	Tyr	Ser	Val	Val	Arg	Ser	Leu	Ser	290	295	300
Glu	Ala	Pro	Asn	Tyr	Ala	Ser	Ala	Ile	Ala	Tyr	Ile	Leu	Lys	His	Asp	305	310	315
His	Ser	Arg	Gly	Arg	Leu	Thr	His	Glu	Gln	Ser	Asn	Glu	Asn	Ile	Ser	325	330	335
Met	Gln	Ala	Trp	Asn	Thr	Leu	Trp	Pro	Gln	Glu	Arg	Lys	Arg	Gln	Arg	340	345	350
Ala	Phe	Phe	Leu	Phe	Gly	Leu	Ala	Leu	Ile	Leu	Gln	Leu	Asp	Ile	Glu	355	360	365
Gly	Ile	Arg	Thr	Phe	Phe	Arg	Thr	Phe	Phe	Arg	Leu	Pro	Lys	Trp	Met	370	375	380
Trp	His	Gly	Phe	Leu	Gly	Ser	Ser	Leu	Ser	Ser	Ala	Asp	Leu	Ile	Leu	385	390	395
Phe	Ala	Phe	Tyr	Met	Phe	Ile	Ile	Ala	Pro	Asn	Asp	Leu	Arg	Lys	Cys	405	410	415
Leu	Ile	Arg	His	Leu	Val	Ser	Asp	Pro	Thr	Gly	Ala	Thr	Met	Val	Arg	420	425	430

Thr Tyr Leu Thr Leu
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                               Met Leu Pro Phe Leu Ser Ser Leu
                               1           5

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Leu Asn Gly Val Thr Asp Asn Pro Cys Arg Lys Ala Met Asp Thr Leu
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Leu Lys Thr His Asn Lys Leu Glu Phe Leu Pro Gln Val His Gly Ala
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ttg gaa aaa tcc agt agt tta agc tca ttg aag att cag aac cag gag 256
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Phe Ile Lys Ala Ser Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Thr
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Ala Val Ala Gln Gln Val Ser Gly Ala Gly Leu Ser Val Cys Ser Ile
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Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Thr Thr Trp
           155           160           165

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Ser Gly Ala Val Val His Ile Asp Asp Asn Thr Lys Lys Asp Leu Asn
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Gln Lys Cys Ile Thr Asn Gly Val Lys Phe His Gln Ala Lys Val Ile	
205 210 215	
aag gtt att cat gaa gag tcc aaa tct ttg ttg att tgc aat gat ggt	784
Lys Val Ile His Glu Glu Ser Lys Ser Leu Leu Ile Cys Asn Asp Gly	
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Cys Leu Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln Val Ala	
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Tyr Gly Ile Leu Ala Glu Val Glu Gln His Pro Phe Asp Leu Asp Lys	
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Glu Val Trp Lys Asp Trp Trp Pro Ile Glu Arg Arg Gln Arg Glu	
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 Thr Arg Arg Phe Phe Asp Ala Phe Phe Asp Leu Glu Pro Arg Tyr Trp
 460 465 470
 cat ggt ttc tta tca tgc aga ttg ttt ctc ccc gag ctt tta gtt ttt 1552
 His Gly Phe Leu Ser Ser Arg Leu Phe Leu Pro Glu Leu Leu Val Phe
 475 480 485
 ggg ctt tct cta ttc tca cat gcc tct aat act tct agg cta gag atc 1600
 Gly Leu Ser Leu Phe Ser His Ala Ser Asn Thr Ser Arg Leu Glu Ile
 490 495 500
 atg gca aag gga act ctt cct ttg gtt aac atg atc aac aac ttg gta 1648
 Met Ala Lys Gly Thr Leu Pro Leu Val Asn Met Ile Asn Asn Leu Val
 505 510 515 520
 caa gat aca gat taagggtgacc atgatagtta taatgtgctt aataactcat 1700
 Gln Asp Thr Asp
 gcactaatcg tttataaaac acttcaaatt agttttgatg tttatagctt attacatgaa 1760
 ccaaagctta tgatagacgt gctttggtat ttaagagttt cagccaaaaa aaaaaaaaaa 1820
 aaaaaaaaaa 1830
 <210> 34
 <211> 524
 <212> PRT
 <213> Citrus X paradisi
 <400> 34
 Met Leu Pro Phe Leu Ser Ser Leu Leu Asn Gly Val Thr Asp Asn Pro
 1 5 10 15
 Cys Arg Lys Ala Met Asp Thr Leu Leu Lys Thr His Asn Lys Leu Glu
 20 25 30
 Phe Leu Pro Gln Val His Gly Ala Leu Glu Lys Ser Ser Ser Leu Ser
 35 40 45
 Ser Leu Lys Ile Gln Asn Gln Glu Leu Arg Phe Gly Leu Lys Lys Ser
 50 55 60
 Arg Gln Lys Arg Asn Arg Ser Cys Phe Ile Lys Ala Ser Ser Ser Ala
 65 70 75 80
 Leu Leu Glu Leu Val Pro Glu Thr Lys Lys Glu Asn Leu Glu Phe Glu
 85 90 95
 Leu Pro Met Tyr Asp Pro Ser Lys Gly Leu Val Val Asp Leu Ala Val
 100 105 110
 Val Gly Gly Gly Pro Ala Gly Leu Ala Val Ala Gln Gln Val Ser Gly
 115 120 125
 Ala Gly Leu Ser Val Cys Ser Ile Asp Pro Ser Pro Lys Leu Ile Trp
 130 135 140
 Pro Asn Asn Tyr Gly Val Trp Val Asp Glu Phe Glu Ala Met Asp Leu
 145 150 155 160
 Leu Asp Cys Leu Asp Thr Thr Trp Ser Gly Ala Val Val His Ile Asp
 165 170 175
 Asp Asn Thr Lys Lys Asp Leu Asn Arg Pro Tyr Gly Arg Val Asn Arg
 180 185 190

Lys	Leu	Leu	Lys	Ser	Lys	Met	Leu	Gln	Lys	Cys	Ile	Thr	Asn	Gly	Val
	195						200					205			
Lys	Phe	His	Gln	Ala	Lys	Val	Ile	Lys	Val	Ile	His	Glu	Glu	Ser	Lys
	210					215					220				
Ser	Leu	Leu	Ile	Cys	Asn	Asp	Gly	Val	Thr	Ile	Gln	Ala	Ala	Val	Val
225					230					235					240
Leu	Asp	Ala	Thr	Gly	Phe	Ser	Arg	Cys	Leu	Val	Gln	Tyr	Asp	Lys	Pro
				245					250					255	
Tyr	Asn	Pro	Gly	Tyr	Gln	Val	Ala	Tyr	Gly	Ile	Leu	Ala	Glu	Val	Glu
			260					265						270	
Gln	His	Pro	Phe	Asp	Leu	Asp	Lys	Met	Val	Phe	Met	Asp	Trp	Arg	Asp
		275					280					285			
Ser	His	Leu	Asn	Asn	Asn	Ser	Gln	Leu	Lys	Glu	Ala	Asn	Ser	Lys	Ile
	290					295					300				
Pro	Thr	Phe	Leu	Tyr	Ala	Met	Pro	Phe	Ser	Ser	Asn	Arg	Ile	Phe	Leu
305					310						315				320
Glu	Glu	Thr	Ser	Leu	Val	Ala	Arg	Pro	Gly	Val	Pro	Met	Lys	Asp	Ile
				325					330					335	
Gln	Glu	Arg	Met	Val	Ala	Arg	Leu	Lys	His	Leu	Gly	Ile	Lys	Val	Lys
			340				345						350		
Ser	Ile	Glu	Glu	Asp	Glu	His	Cys	Val	Ile	Pro	Met	Gly	Gly	Pro	Leu
	355					360						365			
Pro	Val	Leu	Pro	Gln	Arg	Val	Val	Gly	Ile	Gly	Gly	Thr	Ala	Gly	Met
	370				375						380				
Val	His	Pro	Ser	Thr	Gly	Tyr	Met	Val	Ala	Arg	Thr	Leu	Ala	Ala	Ala
385					390				395						400
Pro	Ile	Val	Ala	Asn	Ala	Ile	Val	Arg	Ser	Leu	Ser	Ser	Asp	Arg	Ser
				405					410					415	
Ile	Ser	Gly	His	Lys	Leu	Ser	Ala	Glu	Val	Trp	Lys	Asp	Leu	Trp	Pro
			420					425					430		
Ile	Glu	Arg	Arg	Arg	Gln	Arg	Glu	Phe	Phe	Cys	Phe	Gly	Met	Asp	Ile
	435						440					445			
Leu	Leu	Lys	Leu	Asp	Leu	Pro	Ala	Thr	Arg	Arg	Phe	Phe	Asp	Ala	Phe
	450					455					460				
Phe	Asp	Leu	Glu	Pro	Arg	Tyr	Trp	His	Gly	Phe	Leu	Ser	Ser	Arg	Leu
465					470					475					480
Phe	Leu	Pro	Glu	Leu	Leu	Val	Phe	Gly	Leu	Ser	Leu	Phe	Ser	His	Ala
				485					490					495	
Ser	Asn	Thr	Ser	Arg	Leu	Glu	Ile	Met	Ala	Lys	Gly	Thr	Leu	Pro	Leu
			500					505						510	
Val	Asn	Met	Ile	Asn	Asn	Leu	Val	Gln	Asp	Thr	Asp				
	515						520								

<210> 35
<211> 787
<212> DNA
<213> Citrus sinensis

<220>

<221> CDS

<222> (2)...(787)

<223> coding for epsilon-cyclase (partial)

<400> 35

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t ctt gct ttg gct gca gaa tca gcg aag ttg gga tta aat gtt gga ctt 49
  Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Trp Gly Leu
    1           5           10           15

att ggc ccg gat ctc cct ttc aca aac aat tat ggt gtg tgg gaa gat 97
  Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp
        20           25           30

gaa ttt aga gat ctt gga ctt gaa ggg tgt atc gaa caa gtc tgg aga 145
  Glu Phe Arg Asp Leu Gly Leu Glu Gly Cys Ile Glu Gln Val Trp Arg
        35           40           45

gac aca gtt gta tat att gat gaa gat gaa ccc atc ttg att ggt cgt 193
  Asp Thr Val Val Tyr Ile Asp Glu Asp Glu Pro Ile Leu Ile Gly Arg
        50           55           60

gct tat gga cga gtt agt cga cat ttg ctt cat gaa gaa tta tta aga 241
  Ala Tyr Gly Arg Val Ser Arg His Leu Leu His Glu Glu Leu Leu Arg
        65           70           75           80

agg tgt gtc gag tca ggt gta tca tat ctt agc tca aaa gtg gaa agc 289
  Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Ser
        85           90           95

att acg gaa tct acc agt ggt cat cgt ctt gta gct tgt gaa cat gat 337
  Ile Thr Glu Ser Thr Ser Gly His Arg Leu Val Ala Cys Glu His Asp
        100          105          110

atg att gtc ccc tgc agg ctt gct act gtt gct tct gga gca gca tca 385
  Met Ile Val Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser
        115          120          125

ggg aag cta ttg gaa tat ggg gtg ggg ggt ccc aaa gtt tct gtc caa 433
  Gly Lys Leu Leu Glu Tyr Gly Val Gly Gly Pro Lys Val Ser Val Gln
        130          135          140

aca gct tat ggt gtg gag gtt gag gtg gaa aat aat cca tat gat cca 481
  Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro
        145          150          155          160

agc ctt atg gtt ttc atg gac tac aga gac tgt act aag caa gaa gtt 529
  Ser Leu Met Val Phe Met Asp Tyr Arg Asp Cys Thr Lys Gln Glu Val
        165          170          175

cca tct ttt gaa tct gac aat cca aca ttt ctt tat gtc atg ccc atg 577
  Pro Ser Phe Glu Ser Asp Asn Pro Thr Phe Leu Tyr Val Met Pro Met
        180          185          190

tct tca aca aga gtt ttc ttt gag gaa act tgt ttg gca tcg aaa gat 625
  Ser Ser Thr Arg Val Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Asp
        195          200          205

ggc tta cgt ttt gac ata ttg aag aaa aag ctc atg gca agg tta gag 673
  Gly Leu Arg Phe Asp Ile Leu Lys Lys Lys Leu Met Ala Arg Leu Glu
        210          215          220

aga ttg gga atc cag gtt ttg aaa act tat gaa gag gaa tgg tca tat 721
  Arg Leu Gly Ile Gln Val Leu Lys Thr Tyr Glu Glu Glu Trp Ser Tyr
        225          230          235          240

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att cca gtt ggt ggt tcc tta cca aat aca gaa caa aga aac ctc gca 769
Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Arg Asn Leu Ala
245 250 255

tat ggt gct gct gct agc 787
Tyr Gly Ala Ala Ala Ser
260

<210> 36
<211> 262
<212> PRT
<213> Citrus sinensis

<400> 36
Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Gly Leu
1 5 10 15
Ile Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp
20 25 30
Glu Phe Arg Asp Leu Gly Leu Glu Gly Cys Ile Glu Gln Val Trp Arg
35 40 45
Asp Thr Val Val Tyr Ile Asp Glu Asp Glu Pro Ile Leu Ile Gly Arg
50 55 60
Ala Tyr Gly Arg Val Ser Arg His Leu Leu His Glu Glu Leu Leu Arg
65 70 75 80
Arg Cys Val Glu Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Ser
85 90 95
Ile Thr Glu Ser Thr Ser Gly His Arg Leu Val Ala Cys Glu His Asp
100 105 110
Met Ile Val Pro Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser
115 120 125
Gly Lys Leu Leu Glu Tyr Gly Val Gly Gly Pro Lys Val Ser Val Gln
130 135 140
Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro
145 150 155 160
Ser Leu Met Val Phe Met Asp Tyr Arg Asp Cys Thr Lys Gln Glu Val
165 170 175
Pro Ser Phe Glu Ser Asp Asn Pro Thr Phe Leu Tyr Val Met Pro Met
180 185 190
Ser Ser Thr Arg Val Phe Phe Glu Glu Thr Cys Leu Ala Ser Lys Asp
195 200 205
Gly Leu Arg Phe Asp Ile Leu Lys Lys Lys Leu Met Ala Arg Leu Glu
210 215 220
Arg Leu Gly Ile Gln Val Leu Lys Thr Tyr Glu Glu Glu Trp Ser Tyr
225 230 235 240
Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Arg Asn Leu Ala
245 250 255
Tyr Gly Ala Ala Ala Ser
260

<210> 37
<211> 2357

<212> DNA

<213> Spinacia oleracea

<220>

<221> CDS

<222> (264)...(1814)

<223> coding for epsilon-cyclase

<400> 37

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cctccctctt ccatggccgc aacccaaacc cagccacctt caccgccgcc gtcgacagca 120
cactgaactt caccactaca aacttaaaaa aaatcttgga gaaatttgat tccgtaaaaa 180
tggagtatta ttgtctcgga gcttcgaaat tcgcaacaat ggcggtttct cctgcgctta 240
atcagacaaa ttgaggaat aaa atg gtt aaa caa cgc cag aat ttc cag acg 293
Met Val Lys Gln Arg Gln Asn Phe Gln Thr
1 5 10

ttt tgc ttt tgg agg ccg aat tct tcg aac gtt gta gta gaa tgt agt 341
Phe Cys Phe Trp Arg Pro Asn Ser Ser Asn Val Val Val Glu Cys Ser
15 20 25

agt cgt agg agt gga agt agt gtt ttg agg agt gcg aat agc gac agt 389
Ser Arg Arg Ser Gly Ser Ser Val Leu Arg Ser Ala Asn Ser Asp Ser
30 35 40

agt tgc gta att gcg cca gag gat ttt gcg aac gaa gaa gat ttc atc 437
Ser Cys Val Ile Ala Pro Glu Asp Phe Ala Asn Glu Glu Asp Phe Ile
45 50 55

aaa gct ggt ggt tcc gag ctt ctt tat gtt caa atg cag cag aat aaa 485
Lys Ala Gly Gly Ser Glu Leu Leu Tyr Val Gln Met Gln Gln Asn Lys
60 65 70

gct atg gat tgt tac tcc aaa att tcc gat aag ctg cgt caa ata tca 533
Ala Met Asp Cys Tyr Ser Lys Ile Ser Asp Lys Leu Arg Gln Ile Ser
75 80 85 90

gat gcc aat gaa ctg ctg gat atg gtg gtt att ggt tgt ggt cca gct 581
Asp Ala Asn Glu Leu Leu Asp Met Val Val Ile Gly Cys Gly Pro Ala
95 100 105

ggt cta gct ttg gct gca gaa tcg gct aaa ctt gga tta aaa gtt ggc 629
Gly Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly Leu Lys Val Gly
110 115 120

ctt gtt ggt cct gat ctt cct ttt acg aat aac tac ggc gtt tgg gaa 677
Leu Val Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu
125 130 135

gat gaa ttc aga gca ttg gga ctt gga ggc tgt atc gag cac gtt tgg 725
Asp Glu Phe Arg Ala Leu Gly Leu Gly Cys Ile Glu His Val Trp
140 145 150

cgt gat acc att gtg tat att gat gat gac aat cct ata tat att ggt 773
Arg Asp Thr Ile Val Tyr Ile Asp Asp Asp Asn Pro Ile Tyr Ile Gly
155 160 165 170

cga tct tat gga aaa gtc agc cgg caa tta ctt cac aag gaa ctg gtg 821
Arg Ser Tyr Gly Lys Val Ser Arg Gln Leu Leu His Lys Glu Leu Val
175 180 185

cac agg tgt ttg gag tca ggt gtc tct tat ctg aat gcg aaa gtg gaa 869
His Arg Cys Leu Glu Ser Gly Val Ser Tyr Leu Asn Ala Lys Val Glu
190 195 200
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aat att atg gaa gga cct gat gga cat agg ctt gtt gct tgt gaa cgt	917
Asn Ile Met Glu Gly Pro Asp Gly His Arg Leu Val Ala Cys Glu Arg	
205 210 215	
ggt gtc act att ccc tgc agg ctt gta act gtt gca tct gga gca gct	965
Gly Val Thr Ile Pro Cys Arg Leu Val Thr Val Ala Ser Gly Ala Ala	
220 225 230	
tca ggg aaa ctt ctg gag tat gaa gtg ggt ggt cca agg gtt tgt gta	1013
Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly Gly Pro Arg Val Cys Val	
235 240 245 250	
caa aca gct tat ggt gtg gag gtg gag gtg gaa aac agt cct tat gat	1061
Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu Asn Ser Pro Tyr Asp	
255 260 265	
ccc aat gtg atg gtg ttc atg gac tac aga gac tac act aaa ctg agc	1109
Pro Asn Val Met Val Phe Met Asp Tyr Arg Asp Tyr Thr Lys Leu Ser	
270 275 280	
gtt caa tct ctg gag gca aag tat cca aca ttc ttg tat gca atg ccg	1157
Val Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu Tyr Ala Met Pro	
285 290 295	
ata tca cca act agg atc ttc ttt gag gag act tgc ttg gct tca gta	1205
Ile Ser Pro Thr Arg Ile Phe Phe Glu Glu Thr Cys Leu Ala Ser Val	
300 305 310	
gat gca atg ccc ttt gac ctg ctc aag aaa aag ctt atg aca aga tta	1253
Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Lys Leu Met Thr Arg Leu	
315 320 325 330	
caa act atg ggt gtt cgt atc acc aaa ata tat gaa gag gag tgg tct	1301
Gln Thr Met Gly Val Arg Ile Thr Lys Ile Tyr Glu Glu Glu Trp Ser	
335 340 345	
tat ata cct gtt ggt ggg tcc tta cca aat aca gag caa aga aac ctt	1349
Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Arg Asn Leu	
350 355 360	
gca ttt ggt gct gct gcg agc atg gtg cat cca gcc aca ggt tat tca	1397
Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser	
365 370 375	
gtc gtg aga tca ctg tca gaa gct cca aag tat gct tct gca att gca	1445
Val Val Arg Ser Leu Ser Glu Ala Pro Lys Tyr Ala Ser Ala Ile Ala	
380 385 390	
aac ttg atc aag aat gac ctg tca aaa aat gca ata ttg cgt cag agg	1493
Asn Leu Ile Lys Asn Asp Leu Ser Lys Asn Ala Ile Leu Arg Gln Arg	
395 400 405 410	
agt gtg ggg aat atc tca atg caa gcc tgg aat act ctt tgg cca caa	1541
Ser Val Gly Asn Ile Ser Met Gln Ala Trp Asn Thr Leu Trp Pro Gln	
415 420 425	
gaa agg aaa cgt cag aga gca ttc ttc ctg ttc gga cta tca ctt ata	1589
Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ser Leu Ile	
430 435 440	
gtc cag ctt gat att gag ggt atc agg aca ttc ttc cgc acc ttc ttc	1637
Val Gln Leu Asp Ile Glu Gly Ile Arg Thr Phe Phe Arg Thr Phe Phe	
445 450 455	

cga gtg cca aaa tgg atg tgg gag gga ttc ctc ggt tct aat ctc tct 1685
 Arg Val Pro Lys Trp Met Trp Glu Gly Phe Leu Gly Ser Asn Leu Ser
 460 465 470

tca gct gat ctc ata ttg ttt gcc ttt tat atg ttc ttt att gct ccg 1733
 Ser Ala Asp Leu Ile Leu Phe Ala Phe Tyr Met Phe Phe Ile Ala Pro
 475 480 485 490

aat gac ttg aga atg ggt ctt ata agg cat cta cta tct gat cct aca 1781
 Asn Asp Leu Arg Met Gly Leu Ile Arg His Leu Leu Ser Asp Pro Thr
 495 500 505

ggg gcg acc atg ata aga acg tac ata aca cta taaaagtaat atgaaatgct 1834
 Gly Ala Thr Met Ile Arg Thr Tyr Ile Thr Leu
 510 515

cactcctttg tacatcatgc aaaattggta cgaattgact ggactatgca gtctaacttg 1894
 gtgtaaaaaa aacacaatta ataaattttt tgtaggtgca gcctctatac ttgatattct 1954
 cgattcagat ataattattgt cagtattctt cgtaaagat cagttgtttc tacaattcca 2014
 gaggtcctg gaattgggtg tacccttcca ttagctcat tgataaatgt tgagggtaga 2074
 ggctttttct tagatgcttg cttgcagctt gctcatggat atattcagtt gttcagtaga 2134
 caggttaaca actactacag tgggggcac attgatctgg accgggagag ctgagcatct 2194
 atcacaggtt agccagctca actacgtagg tcaaccttga gccactccca aacatttttg 2254
 cagctgatgg ggttcaccct gtaaggtagg tttcttacca actccacca cttatgttgg 2314
 ttttaaattg ctactcgtct gttatgaagt agcaagctcg tgc 2357

<210> 38

<211> 517

<212> PRT

<213> Spinacia oleracea

<400> 38

Met Val Lys Gln Arg Gln Asn Phe Gln Thr Phe Cys Phe Trp Arg Pro
 1 5 10 15
 Asn Ser Ser Asn Val Val Val Glu Cys Ser Ser Arg Arg Ser Gly Ser
 20 25 30
 Ser Val Leu Arg Ser Ala Asn Ser Asp Ser Ser Cys Val Ile Ala Pro
 35 40 45
 Glu Asp Phe Ala Asn Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu
 50 55 60
 Leu Leu Tyr Val Gln Met Gln Gln Asn Lys Ala Met Asp Cys Tyr Ser
 65 70 75 80
 Lys Ile Ser Asp Lys Leu Arg Gln Ile Ser Asp Ala Asn Glu Leu Leu
 85 90 95
 Asp Met Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala
 100 105 110
 Glu Ser Ala Lys Leu Gly Leu Lys Val Gly Leu Val Gly Pro Asp Leu
 115 120 125
 Pro Phe Thr Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Arg Ala Leu
 130 135 140
 Gly Leu Gly Gly Cys Ile Glu His Val Trp Arg Asp Thr Ile Val Tyr
 145 150 155 160
 Ile Asp Asp Asp Asn Pro Ile Tyr Ile Gly Arg Ser Tyr Gly Lys Val
 165 170 175

Ser Arg Gln Leu Leu His Lys Glu Leu Val His Arg Cys Leu Glu Ser
180 185 190

Gly Val Ser Tyr Leu Asn Ala Lys Val Glu Asn Ile Met Glu Gly Pro
195 200 205

Asp Gly His Arg Leu Val Ala Cys Glu Arg Gly Val Thr Ile Pro Cys
210 215 220

Arg Leu Val Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu
225 230 235 240

Tyr Glu Val Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val
245 250 255

Glu Val Glu Val Glu Asn Ser Pro Tyr Asp Pro Asn Val Met Val Phe
260 265 270

Met Asp Tyr Arg Asp Tyr Thr Lys Leu Ser Val Gln Ser Leu Glu Ala
275 280 285

Lys Tyr Pro Thr Phe Leu Tyr Ala Met Pro Ile Ser Pro Thr Arg Ile
290 295 300

Phe Phe Glu Glu Thr Cys Leu Ala Ser Val Asp Ala Met Pro Phe Asp
305 310 315 320

Leu Leu Lys Lys Lys Leu Met Thr Arg Leu Gln Thr Met Gly Val Arg
325 330 335

Ile Thr Lys Ile Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly
340 345 350

Ser Leu Pro Asn Thr Glu Gln Arg Asn Leu Ala Phe Gly Ala Ala Ala
355 360 365

Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser
370 375 380

Glu Ala Pro Lys Tyr Ala Ser Ala Ile Ala Asn Leu Ile Lys Asn Asp
385 390 395 400

Leu Ser Lys Asn Ala Ile Leu Arg Gln Arg Ser Val Gly Asn Ile Ser
405 410 415

Met Gln Ala Trp Asn Thr Leu Trp Pro Gln Glu Arg Lys Arg Gln Arg
420 425 430

Ala Phe Phe Leu Phe Gly Leu Ser Leu Ile Val Gln Leu Asp Ile Glu
435 440 445

Gly Ile Arg Thr Phe Phe Arg Thr Phe Phe Arg Val Pro Lys Trp Met
450 455 460

Trp Glu Gly Phe Leu Gly Ser Asn Leu Ser Ser Ala Asp Leu Ile Leu
465 470 475 480

Phe Ala Phe Tyr Met Phe Phe Ile Ala Pro Asn Asp Leu Arg Met Gly
485 490 495

Leu Ile Arg His Leu Leu Ser Asp Pro Thr Gly Ala Thr Met Ile Arg
500 505 510

Thr Tyr Ile Thr Leu
515

<210> 39

<211> 1378

<212> DNA

<213> Solanum tuberosum

<220>

<221> CDS

<222> (2)...(1147)

<223> coding for epsilon-cyclase (partial)

<400> 39

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  Ser Xaa Xaa Xaa Asp Glu Phe Lys Asp Leu Gly Leu Gln Ala Cys Ile
    1           5           10           15

gaa cat gtt tgg cgg gat acc att gta tat ctt gat gat gat gat cct 97
Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Asp Asp Pro
    20           25           30

att ctt att ggc cgt gcc tat gga aga gtt agt cgc cat tta ctg cac 145
Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu His
    35           40           45

gag gag tta ctc aaa agg tgt gtg gag gca ggt gtt ttg tat cta aac 193
Glu Glu Leu Leu Lys Arg Cys Val Glu Ala Gly Val Leu Tyr Leu Asn
    50           55           60

tcg aaa gtg gat agg att gtt gag gcc aca aat ggc cac agt ctt gta 241
Ser Lys Val Asp Arg Ile Val Glu Ala Thr Asn Gly His Ser Leu Val
    65           70           75           80

gag tgc gag ggt gat gtt gtg att ccc tgc agg ttt gtg act gtt gca 289
Glu Cys Glu Gly Asp Val Val Ile Pro Cys Arg Phe Val Thr Val Ala
    85           90           95

tcg gga gca gcc tcg ggg aaa ttc ttg cag tat gag ttg gga ggt cct 337
Ser Gly Ala Ala Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Gly Pro
   100           105           110

aga gtt tct gtt caa aca gct tat gga gtg gaa gtt gag gtc gat aac 385
Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Asp Asn
   115           120           125

aat cca ttt gac ccg agc ctg atg gtt ttc atg gat tat aga gac tat 433
Asn Pro Phe Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr
   130           135           140

gtc aga cac gac gct caa tct tta gaa gct aaa tat cca aca ttt ctc 481
Val Arg His Asp Ala Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu
   145           150           155           160

tat gcc atg ccc atg tct cca aca cga gtc ttt ttc gag gaa act tgt 529
Tyr Ala Met Pro Met Ser Pro Thr Arg Val Phe Phe Glu Glu Thr Cys
   165           170           175

ttg gct tca aaa gat gca atg cca ttc gat ctg tta aag aaa aaa ttg 577
Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Lys Leu
   180           185           190

atg tta cga ttg aac acc ctc ggt gta aga att aaa gaa att tat gag 625
Met Leu Arg Leu Asn Thr Leu Gly Val Arg Ile Lys Glu Ile Tyr Glu
   195           200           205

gag gaa tgg tct tac ata cca gtt gga gga tct ttg cca aat aca gaa 673
Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu
   210           215           220

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caa aaa aca ctt gca ttt ggt gct gct gct agc atg gtt cat cca gcc 721
Gln Lys Thr Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala
225                230                235                240

aca ggt tat tca gtc gtc aga tca ctg tct gaa gct cca aaa tgc gcc 769
Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Cys Ala
                245                250                255

ttc gtg ctt gca aat ata tta cga caa aat cat agc aag aat atg ctt 817
Phe Val Leu Ala Asn Ile Leu Arg Gln Asn His Ser Lys Asn Met Leu
                260                265                270

act agt tca agt acc ccg agt att tca act caa gct tgg aac act ctt 865
Thr Ser Ser Ser Thr Pro Ser Ile Ser Thr Gln Ala Trp Asn Thr Leu
                275                280                285

tgg cca caa gaa cga aaa cga caa aga tcg ttt ttc cta ttt gga ctg 913
Trp Pro Gln Glu Arg Lys Arg Gln Arg Ser Phe Phe Leu Phe Gly Leu
                290                295                300

gct ctg ata ttg cag ctg gat att gag ggg ata agg tca ttt ttc cgc 961
Ala Leu Ile Leu Gln Leu Asp Ile Glu Gly Ile Arg Ser Phe Phe Arg
305                310                315                320

gcg ttc ttc cgt gtg cca aaa tgg atg tgg cag gga ttt ctt ggt tca 1009
Ala Phe Phe Arg Val Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser
                325                330                335

agt ctt tct tna gca gac ctc atg tta ttt gcc ttc tac atg ttt att 1057
Ser Leu Ser Xaa Ala Asp Leu Met Leu Phe Ala Phe Tyr Met Phe Ile
                340                345                350

att gca cca aat gac atg aga aga ggc tta atc aga cat ctt tta tct 1105
Ile Ala Pro Asn Asp Met Arg Arg Gly Leu Ile Arg His Leu Leu Ser
                355                360                365

gat cct act ggt gca aca ttg ata aga act tat ctt aca ttt 1147
Asp Pro Thr Gly Ala Thr Leu Ile Arg Thr Tyr Leu Thr Phe
                370                375                380

tagagtaaatt tcctcctaca atagttgttg aannagaggc ctcattactt cagattcata 1207
acagaaatcg cgggtctctcg aggccttgta tataacattt tcactagggtt aatattgctt 1267
gaataagttg cacagtttca gtttttgtat ctgcttcttt tttgtccaag atcatgtatt 1327
ganccaattt atatacattg ccagtatatata taaattttat aaaaaaaaaa a 1378

<210> 40
<211> 382
<212> PRT
<213> Solanum tuberosum

<400> 40
Ser Xaa Xaa Xaa Asp Glu Phe Lys Asp Leu Gly Leu Gln Ala Cys Ile
 1                5                10                15
Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Asp Asp Pro
                20                25                30
Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu His
                35                40                45
Glu Glu Leu Leu Lys Arg Cys Val Glu Ala Gly Val Leu Tyr Leu Asn
                50                55                60
Ser Lys Val Asp Arg Ile Val Glu Ala Thr Asn Gly His Ser Leu Val
        65                70                75                80

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Glu Cys Glu Gly Asp Val Val Ile Pro Cys Arg Phe Val Thr Val Ala
 85 90 95
 Ser Gly Ala Ala Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Gly Pro
 100 105 110
 Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Asp Asn
 115 120 125
 Asn Pro Phe Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr
 130 135 140
 Val Arg His Asp Ala Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe Leu
 145 150 155 160
 Tyr Ala Met Pro Met Ser Pro Thr Arg Val Phe Phe Glu Glu Thr Cys
 165 170 175
 Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Lys Leu
 180 185 190
 Met Leu Arg Leu Asn Thr Leu Gly Val Arg Ile Lys Glu Ile Tyr Glu
 195 200 205
 Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu
 210 215 220
 Gln Lys Thr Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro Ala
 225 230 235 240
 Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Cys Ala
 245 250 255
 Phe Val Leu Ala Asn Ile Leu Arg Gln Asn His Ser Lys Asn Met Leu
 260 265 270
 Thr Ser Ser Ser Thr Pro Ser Ile Ser Thr Gln Ala Trp Asn Thr Leu
 275 280 285
 Trp Pro Gln Glu Arg Lys Arg Gln Arg Ser Phe Phe Leu Phe Gly Leu
 290 295 300
 Ala Leu Ile Leu Gln Leu Asp Ile Glu Gly Ile Arg Ser Phe Phe Arg
 305 310 315 320
 Ala Phe Phe Arg Val Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser
 325 330 335
 Ser Leu Ser Xaa Ala Asp Leu Met Leu Phe Ala Phe Tyr Met Phe Ile
 340 345 350
 Ile Ala Pro Asn Asp Met Arg Arg Gly Leu Ile Arg His Leu Leu Ser
 355 360 365
 Asp Pro Thr Gly Ala Thr Leu Ile Arg Thr Tyr Leu Thr Phe
 370 375 380

<210> 41
 <211> 497
 <212> DNA
 <213> Daucus carota
 <220>
 <221> CDS
 <222> (1)...(495)
 <223> coding for epsilon-cyclase (partial)

<400> 41

tat ggt gtt tgg gtg gat gaa ttt ata gat ctt gga ctt gaa ggg tgt	48
Tyr Gly Val Trp Val Asp Glu Phe Ile Asp Leu Gly Leu Glu Gly Cys	
1 5 10 15	
att gag cat gtt tgg cgg gat act att gta tat ctt gat gat ggt gat	96
Ile Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Gly Asp	
20 25 30	
cct att atg att ggc cgt gct tac gga aga gtt agt cgc cat ttg ctt	144
Pro Ile Met Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu	
35 40 45	
cat gaa gaa ttg ctt aaa agg tgt gtc gag tca ggt gtt tcg tat ctt	192
His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr Leu	
50 55 60	
agc tca aaa gtt gaa aag att att gaa gct gga gat ggc cac agc ctg	240
Ser Ser Lys Val Glu Lys Ile Ile Glu Ala Gly Asp Gly His Ser Leu	
65 70 75 80	
gtt gag tgt gaa aat aat att gtc att cca tgc agg ctt gct act gtt	288
Val Glu Cys Glu Asn Asn Ile Val Ile Pro Cys Arg Leu Ala Thr Val	
85 90 95	
gca tct gga gca gct tct ggg aaa ctt ttg cag tat gag gtt ggg ggt	336
Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val Gly Gly	
100 105 110	
ccc aga gtt tct gtc caa aca gct tat ggt gtc gag gtt gag gtg gaa	384
Pro Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu	
115 120 125	
aac aat cca tat gat ccc agt cta atg gtt ttc atg gat tac aga gat	432
Asn Asn Pro Tyr Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp	
130 135 140	
tat acc aaa caa aaa gtt cca ggc atg gag gca gaa tat cca act ttc	480
Tyr Thr Lys Gln Lys Val Pro Gly Met Glu Ala Glu Tyr Pro Thr Phe	
145 150 155 160	
ctg tat gcc atg cca tt	497
Leu Tyr Ala Met Pro	
165	

<210> 42

<211> 165

<212> PRT

<213> Daucus carota

<400> 42

Tyr Gly Val Trp Val Asp Glu Phe Ile Asp Leu Gly Leu Glu Gly Cys	
1 5 10 15	
Ile Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Gly Asp	
20 25 30	
Pro Ile Met Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu Leu	
35 40 45	
His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr Leu	
50 55 60	
Ser Ser Lys Val Glu Lys Ile Ile Glu Ala Gly Asp Gly His Ser Leu	
65 70 75 80	

Val Glu Cys Glu Asn Asn Ile Val Ile Pro Cys Arg Leu Ala Thr Val
85 90 95
Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val Gly Gly
100 105 110
Pro Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Glu
115 120 125
Asn Asn Pro Tyr Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp
130 135 140
Tyr Thr Lys Gln Lys Val Pro Gly Met Glu Ala Glu Tyr Pro Thr Phe
145 150 155 160
Leu Tyr Ala Met Pro
165

<210> 43
<211> 605
<212> DNA
<213> Daucus carota
<220>
<221> CDS
<222> (3)...(605)
<223> coding for episilon-cyclase (partial)
<400> 43

tc att ggc cgt gct tat gga aga tta gtc gcc att tgc ttc atg aag 47
Ile Gly Arg Ala Tyr Gly Arg Leu Val Ala Ile Cys Phe Met Lys
1 5 10 15
aat tgc tta aaa ggt gtg tgc agt cag gtg ttt cgt atc tta gct caa 95
Asn Cys Leu Lys Gly Val Ser Ser Gln Val Phe Arg Ile Leu Ala Gln
20 25 30
aag ttg aaa aga tta ttg aag ctg gag atg gcc aca gcc tgg ttg agt 143
Lys Leu Lys Arg Leu Leu Lys Leu Glu Met Ala Thr Ala Trp Leu Ser
35 40 45
gtg aaa ata ata ttg tca ttc cat gca ggc ttg cta ctg ttg cat ctg 191
Val Lys Ile Ile Leu Ser Phe His Ala Gly Leu Leu Leu Leu His Leu
50 55 60
gag cag ctt ctg gga aac ttt tgc agt atg ggg ttg ggg gtc cca gag 239
Glu Gln Leu Leu Gly Asn Phe Cys Ser Met Gly Leu Gly Val Pro Glu
65 70 75
ttt ctg tcc aaa cag ctt atg gtg tgc agg ttg agg tgg aaa cca atc 287
Phe Leu Ser Lys Gln Leu Met Val Ser Arg Leu Arg Trp Lys Pro Ile
80 85 90 95
cca tat gat ccc agt cta atg gtt ttc atg gat tac aga gat tat acc 335
Pro Tyr Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Thr
100 105 110
aaa caa aaa gtt cca ggc atg gag gca gaa tat cca aca ttt ctt tat 383
Lys Gln Lys Val Pro Gly Met Glu Ala Glu Tyr Pro Thr Phe Leu Tyr
115 120 125
gtg atg ccc atg tcc cca aca agg att ttc ttt gag gag aca tgt ttg 431
Val Met Pro Met Ser Pro Thr Arg Ile Phe Phe Glu Glu Thr Cys Leu
130 135 140

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gct tca aaa gat gcg atg cca ttc gat cta ctg aag aaa aaa ctc atg 479
Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Lys Leu Met
145 150 155

tca aga tta cag acg atg gga att cga gtt gcc aag aca tat gaa gag 527
Ser Arg Leu Gln Thr Met Gly Ile Arg Val Ala Lys Thr Tyr Glu Glu
160 165 170 175

gaa tgg tct tat ata cct gtt ggg gga tct tta cct aat act gag caa 575
Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln
180 185 190

aag aat ctc gcc ttt ggt gct gcc gct aga 605
Lys Asn Leu Ala Phe Gly Ala Ala Ala Arg
195 200

```

<210> 44
<211> 201
<212> PRT
<213> Daucus carota

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<400> 44
Ile Gly Arg Ala Tyr Gly Arg Leu Val Ala Ile Cys Phe Met Lys Asn
1 5 10 15

Cys Leu Lys Gly Val Ser Ser Gln Val Phe Arg Ile Leu Ala Gln Lys
20 25 30

Leu Lys Arg Leu Leu Lys Leu Glu Met Ala Thr Ala Trp Leu Ser Val
35 40 45

Lys Ile Ile Leu Ser Phe His Ala Gly Leu Leu Leu Leu His Leu Glu
50 55 60

Gln Leu Leu Gly Asn Phe Cys Ser Met Gly Leu Gly Val Pro Glu Phe
65 70 75 80

Leu Ser Lys Gln Leu Met Val Ser Arg Leu Arg Trp Lys Pro Ile Pro
85 90 95

Tyr Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp Tyr Thr Lys
100 105 110

Gln Lys Val Pro Gly Met Glu Ala Glu Tyr Pro Thr Phe Leu Tyr Val
115 120 125

Met Pro Met Ser Pro Thr Arg Ile Phe Phe Glu Glu Thr Cys Leu Ala
130 135 140

Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Lys Leu Met Ser
145 150 155 160

Arg Leu Gln Thr Met Gly Ile Arg Val Ala Lys Thr Tyr Glu Glu Glu
165 170 175

Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr Glu Gln Lys
180 185 190

Asn Leu Ala Phe Gly Ala Ala Ala Arg
195 200

```

<210> 45
<211> 1697
<212> DNA
<213> Lycopersicon esculentum

<220>

<221> CDS

<222> (6)..(1583)

<223> coding for epsilon-cyclase

<400> 45

ttgaa atg gag tgt gtt gga gtt caa aat gtt gga gca atg gca gtt tta 50

Met Glu Cys Val Gly Val Gln Asn Val Gly Ala Met Ala Val Leu

1

5

10

15

acg cgt ccg aga ttg aac cgt tgg tcg gga gga gag tta tgc caa gaa 98

Thr Arg Pro Arg Leu Asn Arg Trp Ser Gly Gly Glu Leu Cys Gln Glu

20

25

30

aaa agc atc ttt ttg gcg tat gag cag tat gaa agt aaa tgt aat agc 146

Lys Ser Ile Phe Leu Ala Tyr Glu Gln Tyr Glu Ser Lys Cys Asn Ser

35

40

45

agt agt ggt agt gac agt tgt gta gtt gat aaa gaa gat ttt gct gat 194

Ser Ser Gly Ser Asp Ser Cys Val Val Asp Lys Glu Asp Phe Ala Asp

50

55

60

gaa gaa gat tat ata aaa gcc ggt ggt tcg caa ctt gta ttt gtt caa 242

Glu Glu Asp Tyr Ile Lys Ala Gly Gly Ser Gln Leu Val Phe Val Gln

65

70

75

atg cag cag aaa aaa gat atg gat cag cag tct aag ctt tct gat gag 290

Met Gln Gln Lys Lys Asp Met Asp Gln Gln Ser Lys Leu Ser Asp Glu

80

85

90

95

tta cga caa ata tct gct gga caa acc gta ctg gat tta gtg gta atc 338

Leu Arg Gln Ile Ser Ala Gly Gln Thr Val Leu Asp Leu Val Val Ile

100

105

110

ggc tgt ggt cct gct ggt ctt gct ctt gcc gcg gag tca gct aaa ttg 386

Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu

115

120

125

ggg ttg aac gtg ggg ctg gtt ggg cct gat ctt cct ttc aca aac aac 434

Gly Leu Asn Val Gly Leu Val Gly Pro Asp Leu Pro Phe Thr Asn Asn

130

135

140

tat ggt gta tgg gag gac gag ttc aaa gat ctt ggt ctt caa gcc tgc 482

Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Gln Ala Cys

145

150

155

att gaa cat gtt tgg cgg gat acc att gta tat ctt gat gat gat gaa 530

Ile Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Asp Glu

160

165

170

175

cct att ctt att ggc cgt gcc tat gga aga gtt agt cgc cat ttt ctg 578

Pro Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Phe Leu

180

185

190

cac gag gag tta ctg aaa agg tgt gtg gag gca ggt gtt ttg tat cta 626

His Glu Glu Leu Leu Lys Arg Cys Val Glu Ala Gly Val Leu Tyr Leu

195

200

205

aac tcg aaa gtg gat agg att gtt gag gcc aca aat ggc cag agt ctt 674

Asn Ser Lys Val Asp Arg Ile Val Glu Ala Thr Asn Gly Gln Ser Leu

210

215

220

gta gag tgc gaa ggt gat gtt gtg att ccc tgc agg ttt gtg act gtt 722

Val Glu Cys Glu Gly Asp Val Val Ile Pro Cys Arg Phe Val Thr Val

225

230

235

gca tcg ggg gca gcc tcg ggg aaa ttc ttg cag tat gag ttg gga agt	770
Ala Ser Gly Ala Ala Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Ser	
240 245 250 255	
cct aga gtt tct gtt caa aca gct tat gga gtg gaa gtt gag gtt gat	818
Pro Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Asp	
260 265 270	
aac aat cca ttt gac ccg agc ctg atg gtt ttc atg gat tat aga gat	866
Asn Asn Pro Phe Asp Pro Ser Leu Met Val Phe Met Asp Tyr Arg Asp	
275 280 285	
tat ctc aga cac gac gct caa tct tta gaa gct aaa tat cca aca ttt	914
Tyr Leu Arg His Asp Ala Gln Ser Leu Glu Ala Lys Tyr Pro Thr Phe	
290 295 300	
ctt tat gcc atg ccc atg tct cca aca cga gtc ttt ttc gag gaa act	962
Leu Tyr Ala Met Pro Met Ser Pro Thr Arg Val Phe Phe Glu Glu Thr	
305 310 315	
tgt ttg gct tca aaa gat gca atg cca ttc gat ctg tta aag aaa aaa	1010
Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Lys Lys	
320 325 330 335	
ctg atg cta cga ttg aac acc ctt ggt gta aga att aaa gaa att tac	1058
Leu Met Leu Arg Leu Asn Thr Leu Gly Val Arg Ile Lys Glu Ile Tyr	
340 345 350	
gag gag gaa tgg tct tac ata ccg gtt ggt gga tct ttg cca aat aca	1106
Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn Thr	
355 360 365	
gaa caa aaa aca ctt gca ttt ggt gct gct gct agc atg gtt cat cca	1154
Glu Gln Lys Thr Leu Ala Phe Gly Ala Ala Ala Ser Met Val His Pro	
370 375 380	
gcc aca ggt tat tca gtc gtc aga tca ctt tct gaa gct cca aaa tgc	1202
Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys Cys	
385 390 395	
gcc tct gta ctt gca aat ata tta cga caa cat tat agc aag aac atg	1250
Ala Ser Val Leu Ala Asn Ile Leu Arg Gln His Tyr Ser Lys Asn Met	
400 405 410 415	
ctt acc agt tca agt atc ccg agt ata tca act caa gct tgg aac act	1298
Leu Thr Ser Ser Ser Ile Pro Ser Ile Ser Thr Gln Ala Trp Asn Thr	
420 425 430	
ctt tgg cca caa gaa cga aaa cga caa aga tcg ttt ttc cta ttt gga	1346
Leu Trp Pro Gln Glu Arg Lys Arg Gln Arg Ser Phe Phe Leu Phe Gly	
435 440 445	
ctg gct ctg ata ttg cag ctg gat att gag ggg ata agg tca ttt ttc	1394
Leu Ala Leu Ile Leu Gln Leu Asp Ile Glu Gly Ile Arg Ser Phe Phe	
450 455 460	
cgc gca ttc ttc cgt gtg cca aaa tgg atg tgg cag gga ttt ctt ggt	1442
Arg Ala Phe Phe Arg Val Pro Lys Trp Met Trp Gln Gly Phe Leu Gly	
465 470 475	
tca agt ctt tct tca gca gac ctc atg tta ttt gcc ttc tac atg ttt	1490
Ser Ser Leu Ser Ser Ala Asp Leu Met Leu Phe Ala Phe Tyr Met Phe	
480 485 490 495	

att att gca cca aat gac atg aga aaa ggc ttg atc aga cat ctt tta 1538
 ile ile ala pro asn asp met arg lys gly leu ile arg his leu leu
 500 505 510

tct gat cct act ggt gca aca ttg ata aga act tat ctt aca ttt 1583
 ser asp pro thr gly ala thr leu ile arg thr tyr leu thr phe
 515 520 525

tagagtaaac tcctcctaca ataattgttg aatcagaggc ctcattactt cagattcata 1643
 acagaaatcg cggctctctcg aggcattgta tataacattt tcactagctt aata 1697

<210> 46

<211> 526

<212> PRT

<213> *Lycopersicon esculentum*

<400> 46

Met Glu Cys Val Gly Val Gln Asn Val Gly Ala Met Ala Val Leu Thr
 1 5 10 15
 Arg Pro Arg Leu Asn Arg Trp Ser Gly Gly Glu Leu Cys Gln Glu Lys
 20 25 30
 Ser Ile Phe Leu Ala Tyr Glu Gln Tyr Glu Ser Lys Cys Asn Ser Ser
 35 40 45
 Ser Gly Ser Asp Ser Cys Val Val Asp Lys Glu Asp Phe Ala Asp Glu
 50 55 60
 Glu Asp Tyr Ile Lys Ala Gly Gly Ser Gln Leu Val Phe Val Gln Met
 65 70 75 80
 Gln Gln Lys Lys Asp Met Asp Gln Gln Ser Lys Leu Ser Asp Glu Leu
 85 90 95
 Arg Gln Ile Ser Ala Gly Gln Thr Val Leu Asp Leu Val Val Ile Gly
 100 105 110
 Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala Glu Ser Ala Lys Leu Gly
 115 120 125
 Leu Asn Val Gly Leu Val Gly Pro Asp Leu Pro Phe Thr Asn Asn Tyr
 130 135 140
 Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Gln Ala Cys Ile
 145 150 155 160
 Glu His Val Trp Arg Asp Thr Ile Val Tyr Leu Asp Asp Asp Glu Pro
 165 170 175
 Ile Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Phe Leu His
 180 185 190
 Glu Glu Leu Leu Lys Arg Cys Val Glu Ala Gly Val Leu Tyr Leu Asn
 195 200 205
 Ser Lys Val Asp Arg Ile Val Glu Ala Thr Asn Gly Gln Ser Leu Val
 210 215 220
 Glu Cys Glu Gly Asp Val Val Ile Pro Cys Arg Phe Val Thr Val Ala
 225 230 235 240
 Ser Gly Ala Ala Ser Gly Lys Phe Leu Gln Tyr Glu Leu Gly Ser Pro
 245 250 255
 Arg Val Ser Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val Asp Asn
 260 265 270

Asn	Pro	Phe	Asp	Pro	Ser	Leu	Met	Val	Phe	Met	Asp	Tyr	Arg	Asp	Tyr
		275					280					285			
Leu	Arg	His	Asp	Ala	Gln	Ser	Leu	Glu	Ala	Lys	Tyr	Pro	Thr	Phe	Leu
	290					295					300				
Tyr	Ala	Met	Pro	Met	Ser	Pro	Thr	Arg	Val	Phe	Phe	Glu	Glu	Thr	Cys
305					310					315					320
Leu	Ala	Ser	Lys	Asp	Ala	Met	Pro	Phe	Asp	Leu	Leu	Lys	Lys	Lys	Leu
				325					330						335
Met	Leu	Arg	Leu	Asn	Thr	Leu	Gly	Val	Arg	Ile	Lys	Glu	Ile	Tyr	Glu
			340					345					350		
Glu	Glu	Trp	Ser	Tyr	Ile	Pro	Val	Gly	Gly	Ser	Leu	Pro	Asn	Thr	Glu
		355					360					365			
Gln	Lys	Thr	Leu	Ala	Phe	Gly	Ala	Ala	Ala	Ser	Met	Val	His	Pro	Ala
	370					375					380				
Thr	Gly	Tyr	Ser	Val	Val	Arg	Ser	Leu	Ser	Glu	Ala	Pro	Lys	Cys	Ala
385					390					395					400
Ser	Val	Leu	Ala	Asn	Ile	Leu	Arg	Gln	His	Tyr	Ser	Lys	Asn	Met	Leu
				405					410					415	
Thr	Ser	Ser	Ser	Ile	Pro	Ser	Ile	Ser	Thr	Gln	Ala	Trp	Asn	Thr	Leu
			420					425					430		
Trp	Pro	Gln	Glu	Arg	Lys	Arg	Gln	Arg	Ser	Phe	Phe	Leu	Phe	Gly	Leu
		435					440					445			
Ala	Leu	Ile	Leu	Gln	Leu	Asp	Ile	Glu	Gly	Ile	Arg	Ser	Phe	Phe	Arg
	450					455					460				
Ala	Phe	Phe	Arg	Val	Pro	Lys	Trp	Met	Trp	Gln	Gly	Phe	Leu	Gly	Ser
465					470					475					480
Ser	Leu	Ser	Ser	Ala	Asp	Leu	Met	Leu	Phe	Ala	Phe	Tyr	Met	Phe	Ile
				485					490					495	
Ile	Ala	Pro	Asn	Asp	Met	Arg	Lys	Gly	Leu	Ile	Arg	His	Leu	Leu	Ser
			500					505					510		
Asp	Pro	Thr	Gly	Ala	Thr	Leu	Ile	Arg	Thr	Tyr	Leu	Thr	Phe		
		515					520					525			

<210> 47

<211> 510

<212> DNA

<213> Tagetes erecta

<220>

<221> misc feature

$\langle 222 \rangle$ (1) . . (510)

<223> coding for epsilon-cyclase specific probe

<400> 47

ggcagcaggc	aaagcaagg	ttgtttgttg	ttgttggtga	gagacactcc	aatccaaaca	60
gatacaaggc	gtgactggat	atttctctct	cgttcctaac	aacagcaacg	aagaagaaaa	120
agaatcatta	ctaacaatca	atgagtatga	gagctggaca	catgacggca	acaatggcgg	180
cttttacatg	ccctagggtt	atgactagca	tcagatacac	gaagcaaatt	aagtgc AACG	240
ctgctaaaag	ccagctagtc	gttaaacaa	agattgagga	ggaagaagat	tatgtgaaag	300
cgggtggatc	ggagctgctt	tttgttcaaa	tgcaacagaa	taagtccatg	gatgcacagt	360
ctagcctatc	ccaaaagctc	ccaagggtag	caataggagg	taaggaggag	agtaactgtg	420

tactggattt ggttgtaatt ggttggtgct ctgctggcct tgctcttgct ggagaatcag 480
ccaagctagg ctggaatgct gcacttatcg 510

<210> 48

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
oligonucleotide primer

<400> 48

ggcacgaggg aaagcaaagg

20

<210> 49

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
oligonucleotide primer

<400> 49

cgataagtgc gacattcaag c

21

<210> 50

<211> 734

<212> DNA

<213> Tagetes erecta

<220>

<221> misc_feature

<222> (1) .. (734)

<223> fragment of epsilon cyclase gene obtain by iPCR
comprising part of promoter region

<400> 50

ctaacaatca atgagtagag agctggacac atgacggcaa caatggcggc ttttacatgc 60
cctaggttta tgactagcat cagatacacg aagcaaatta agtgcaacgc tgctaaaagc 120
cagctagtcg ttaaacaaga gattgaggag gaagaagatt atgtgaaagc cgggtggatcg 180
gagctgcttt ttgttcaa at gcaacagaat aagtccatgg atgcacagtc tagcctatcc 240
caaaaggcca ctccagactt aattgcttat aaataaataa atatgttttt taggaataat 300
gatatttaga tagattagct atcacctgtg ctgtggtgtg cagctcccaa gggctcttacc 360
gatagtaaaa tcgttagtta tgattaatac ttgggaggtg ggggattata ggctttgttg 420
tgagaatggt gagaaagagg ttgacaaat cgggtgttga atgagggtta atggagttta 480
attaaaataa agagaagaga aagattaaga ggggtgatgg gatattaaag acggscata 540
tagtgatgcc acgtagaaaa aggtaagtga aaacatacaa cgtggcttta aaagatggct 600
tggctgctaa tcaactcaac tcaactcata tcctatccat tcaaattcaa ttcaattcta 660
ttgaatgcaa agcaaagcaa aggttggttg ttgttggttg tgagagacac tccaatccaa 720
acagatacaa ggcg 734

<210> 51

<211> 280

<212> DNA

<213> Tagetes erecta

<220>

<221> misc_feature

<222> (1) .. (280)

<223> fragment of epsilon cyclase gene obtain by
TAIL-PCR comprising part of promoter region

<400> 51
gtcgagtatg gagttcaatt aaaataaaga gaagaraaag attaagaggg tgatggggat 60
attaaagacg gccaatrtag tgatgccacg taagaaaaag gtaagtgaaa acatacaacg 120
tggctttaa agatgggcttg gctgctaate aactcaactc aactcatatc ctatccattc 180
aaattcaatt caattctatt gaatgcaaag caaagcaaag caaagggtgt ttgttggtgt 240
tgttgagaga cactccaatc caaacagata caaggcgtga 280

<210> 52
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<223> Description of the artificial sequence:
oligonucleotide primer

<400> 52
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<210> 53
<211> 24
<212> DNA
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<223> Description of the artificial sequence:
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<400> 53
ctaacaatca atgagtatga gagg 24

<210> 54
<211> 26
<212> DNA
<213> Artificial sequence
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<223> Description of the artificial sequence:
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<400> 54
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<210> 55
<211> 26
<212> DNA
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<223> Description of the artificial sequence:
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<400> 55
ccttgaggagc ttttgggata ggctag 26

<210> 56
<211> 26
<212> DNA
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<223> Description of the artificial sequence:
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<400> 56
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<210> 57
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<223> Description of the artificial sequence:
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<400> 57
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15

<210> 58
<211> 734
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<213> Tagetes erecta

<220>
<221> misc_feature
<222> (1)..(734)
<223> coding for epsilon-cyclase genomic iPCR-fragment

<400> 58
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cctaggttta tgactagcat cagatacacg aagcaaatta agtgcaacgc tgctaaaagc 120
cagctagtcg ttaaacaaga gattgaggag gaagaagatt atgtgaaagc cgggtggatcg 180
gagctgcttt ttgttcaaat gcaacagaat aagtcctatg atgcacagtc tagcctatcc 240
caaaagggtca ctccagactt aattgcttat aaataaataa atatgttttt taggaataat 300
gatatttaga tagattagct atcacctgtg ctgtggtgtg cagctcccaa gggctcttacc 360
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tgagaatggt gagaaagagg ttgacaaat cggtgtttga atgaggttaa atggagttaa 480
attaaaataa agagaagaga aagattaaga ggggtgatggg gatattaaag acggscaata 540
tagtgatgcc acgtagaaaa aggtaagtga aaacatacaa cgtggccttta aaagatggct 600
tggctgctaa tcaactcaac tcaactcata tcctatccat tcaaattcaa ttcaattcta 660
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acagatacaa ggcg 734

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25

<210> 60
<211> 29
<212> DNA
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<223> Description of the artificial sequence:
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29

<210> 61
<211> 29

<212> DNA
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<210> 62
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<223> Description of the artificial sequence:
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<210> 63
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<212> DNA
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<223> Description of the artificial sequence:
oligonucleotide primer
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<210> 64
<211> 22
<212> DNA
<213> Artificial sequence
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<223> Description of the artificial sequence:
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gcaagctcga cagctacaaa cc 22
<210> 65
<211> 24
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
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<400> 65
gaagcatgca gctagcagcg acag 24
<210> 66
<211> 1795
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence: coding for
ketolase - 35S terminator construct

<400> 66
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aggagaagga gaaggagggt gcaggcagct ctgacgtgtt gcgtacatgg gcgacccagt 120
actcgcttcc gtcagaggag tcagacgcgg cccgcccggg actgaagaat gcctacaagc 180
caccaccttc cgacacaaag ggcatacaca tggcgctagc tgtcatcgge tcctggggccg 240
cagtgttccct ccacgccatt ttccaaatca agcttccgac ctccctggac cagctgcact 300
ggctgcccgt gtcagatgcc acagctcagc tggtagcgg cagcagcagc ctgctgcaca 360
tcgtcgtagt attctttgtc ctggagttcc tgtacacagg cctttttatc accacgcagt 420
atgctatgca tggcaccatc gccatgagaa acaggcagct taatgacttc ttgggcagag 480
tatgcatctc cttgtacgcc tggtttgatt acaacatgct gcaccgcaag cattggggagc 540
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ttgtgcccgt gtttgccagc ttcattgtcc gctacatgtc gatgtggcag ttgcgcgcc 660
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tcattggcggc cgcgcccata ctgtccgcct tccgcttgtt ctactttggc acgtacatgc 780
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tgcactggga gcaccacgc tggccctttg cccctgggtg ggagctgccc aactgcccgc 960
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cttagtatgt atttgatatt gtaaaatact tctatcaata aaatttctaa ttccataaac 1260
caaaatccag tactaaaatc cagatctcct aaagtccta tagatctttg tcgtgaatat 1320
aaaccagaca cgagacgact aaacctggag cccagacgcc gttcgaagct agaagtaccg 1380
cttaggcagg aggccgttag ggaaaagatg ctaaggcagg gttgggttac ttgactcccc 1440
cgtaggtttg gtttaaatat gatgaagtgg acggaaggaa ggagggaagac aaggaaggat 1500
aagggtgcag gccctgtgca aggtaagaag atggaaattt gatagaggta cgctactata 1560
cttatactat acgctaaggg aatgcttgta ttatatacct atacccccta ataaccctt 1620
atcaatttaa gaaataatcc gcataagccc ccgcttaaaa attgggtatca gagccatgaa 1680
taggtctatg accaaaactc aagaggataa aacctcacca aaatacgaaa gagttcttaa 1740
ctctaaagat aaaagatctt tcaagatcaa aactagttcc ctacacaccg atatac 1795

<210> 67

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
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<400> 67

gagctcactc actgatttcc attgcttg

28

<210> 68

<211> 37

<212> DNA

<213> Artificial sequence

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<223> Description of the artificial sequence:
oligonucleotide primer

<400> 68

cgccgttaag tcgatgtccg ttgatttaaa cagtgtc

37

<210> 69

<211> 34

<212> DNA

<213> Artificial sequence

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<400> 69
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34
<210> 70
<211> 25
<212> DNA
<213> Artificial sequence
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<223> Description of the artificial sequence:
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<400> 70
taagctttttt gttgaagaga tttgg
25
<210> 71
<211> 28
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
      oligonucleotide primer
<400> 71
gtcgactacg taagtttctg cttctacc
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<210> 72
<211> 26
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
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<400> 72
ggatccggtg atacctgcac atcaac
26
<210> 73
<211> 28
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
      oligonucleotide primer
<400> 73
aagcttaccg atagtaaaat cgttagtt
28
<210> 74
<211> 31
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
      oligonucleotide primer
<400> 74
ctcgagctta ccgatagtaa aatcgtagt t
31

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<210> 75
 <211> 28
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
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 <400> 75
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 <210> 76
 <211> 28
 <212> DNA
 <213> Artificial sequence
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 <223> Description of the artificial sequence:
 oligonucleotide primer
 <400> 76
 ggatccaaca acaacaaaca acctttgc 28
 <210> 77
 <211> 777
 <212> DNA
 <213> Arabidopsis thaliana
 <220>
 <221> promoter
 <222> (1) .. (777)
 <223> modified version of the AP3 promoter
 <400> 77
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 agcaaaaaga aaaaagagtt tcatcatata tctgatttga tggactggtt ggagtttagga 180
 ccaaacatta tctacaaaca aagacttttc tcttaacttg tgattccttc ttaaacccta 240
 ggggtaatat tctattttcc aaggatcttt agttaaggc aaatccggga aattattgta 300
 atcatttggg gaaacatata aaagatttga gttagatgga agtgacgatt aatccaaaca 360
 tatatatctc tttcttctta ttcccaaat taacagacaa aagtagaata ttggctttta 420
 acaccaatat aaaaacttgc ttcacacctt aacacttttg tttactttag ggtaagtgc 480
 aaaagccaac caaatccacc tgcactgatt tgacgtttac aaacgccgtt aagtcgatgt 540
 ccgttgattt aaacagtgtc ttgtaattaa aaaaatcagt ttacataaat ggaaaattta 600
 tcaactagtt ttcataact tctgaactta ctttccatgg attaggcaat actttccatt 660
 tttagtaact caagtggacc ctttacttct tcaactccat ctctctctt ctatttccact 720
 tctttcttct cattatatct cttgtcctct ccaccaaatc tcttcaaca aaagctt 777
 <210> 78
 <211> 212
 <212> DNA
 <213> Solanum tuberosum
 <220>
 <221> intron
 <222> (1) .. (212)
 <223> PIV2 intron of ST-LS1 gene
 <400> 78
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 gtagtaatat aatatttcaa atattttttt caaaataaaa gaatgtagta tatagcaatt 120

gcttttctgt agtttataag tgtgtatatt ttaatttata acttttctaa tatatgacca 180
aaatttggtg atgtgcaggt atcacaggat cc 212

<210> 79

<211> 358

<212> DNA

<213> *Tagetes erecta*

<220>

<221> misc_feature

<222> (1)..(358)

<223> coding for sense-strand of epsilon cyclase
promoter directed dsRNA

<400> 79

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gctttgttgt gagaatggtg agaaagaggt ttgacaaatc ggtggttgaa tgaggttaaa 120
tggagtttaa ttaaaataaa gagaagagaa agattaagag ggtgatgggg atattaaaga 180
cggccaatat agtgatgcca cgtagaaaaa ggtaagtga aacatacaac gtggctttaa 240
aagatggctt ggctgctaact caactcaact caactcatat cctatccatt caaattcaat 300
tcaattctat tgaatgcaaa gcaaagcaaa gcaaaggttg tttgttggtg ttgtcgac 358

<210> 80

<211> 361

<212> DNA

<213> *Tagetes erecta*

<220>

<221> misc_feature

<222> (1)..(361)

<223> coding for antisense-strand of epsilon cyclase
promoter directed dsRNA

<400> 80

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aaatggaggt taattaaaat aaagagaaga gaaagattaa gaggggatg gggatattaa 180
agacggccaa tatagtgtg ccacgtagaa aaaggtaagt gaaaacatac aacgtggctt 240
taaaagatgg cttggctgct aatcaactca actcaactca tatectatcc attcaaattc 300
aattcaattc tattgaatgc aaagcaaagc aaagcaaagg ttgtttgttg ttgttgatc 360
c 361

<210> 81

<211> 1537

<212> DNA

<213> *Cucumis sativus*

<220>

<221> promoter

<222> (1)..(1537)

<223> promoter of chromoplast-specific
carotenoid-associated protein (CHRC)

<400> 81

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tgtacattta ttcaataata ttatatgttt attacaaatt ctcaactttct tattcatacc 120
tattcactca agcctttacc atcttctttt tctatttcaa tactattttct acttcatttt 180
tcacgttttt aacatctttc tttattttct gtccacttcg tttagggatg cctaattgtcc 240
caaatttcat ctctcgtagt aacacaaaac caatgtaatg ctacttctct ctacattttt 300
aatacaata aagtgaacaa aaatatctat aaataaacia atatatatat tttgttagac 360
gctgtctcaa cccatcaatt aaaaaatttt gttatatatt tactttacct actaaatttg 420
tttctcatat ttacctttta acccccacaa aaaaaaatta taaaaaagaa agaaaaaagc 480

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taaaccctat ttaaataagct aactataaga tcttaaaaatt atcctcatca gtgtatagtt 540
taattgggta ttaacttata acattatata tctatgacat atactctctc ctagctattt 600
ctcacatttt ttaacttaag aaaatagtc taacatagtc taaaattcaa acatccacat 660
gctctaattt gattaacaaa agtttagaaa tatttattta aataaaaaag actaataaat 720
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<210> 82

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
oligonucleotide primer

<400> 82

gagctctaca aattaggggtt ac

22

<210> 83

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
oligonucleotide primer

<400> 83

aagcttatta ttccaaatt ccg

23

We claim:

1. A method for the transgenic expression of nucleic acid sequences in the flower of plants, including the following steps
 - I. introduction of a transgenic expression cassette into plant cells, where the transgenic expression cassette comprises at least the following elements
 - a) at least one promoter sequence of a gene coding for an ϵ -cyclase, and
 - 10 b) at least one further nucleic acid sequence, and
 - c) where appropriate further genetic control elements;where at least one of said promoter sequences and one further nucleic acid sequence are functionally linked together, and the further nucleic acid sequence is heterologous in relation to the promoter sequence or the plant cell, and
 - II. selection of transgenic cells which comprise said expression cassette stably integrated into the genome, and
 - 20 III. regeneration of complete plants from said transgenic cells, where at least one of the further nucleic acid sequences is expressed in the flower.
2. The method as claimed in claim 1, where the promoter sequence of a gene coding for an ϵ -cyclase is a sequence selected from the group of sequences consisting of
 - 30 i) the promoter sequence of the ϵ -cyclase from *Tagetes erecta* as shown in SEQ ID NO: 1, the ϵ -cyclase from *Arabidopsis thaliana* as shown in SEQ ID NO: 7, the ϵ -cyclase from *Oryza sativa* as shown in SEQ ID NO: 8, and
 - ii) functional equivalents of the promoter sequences as shown in SEQ ID NO: 1, 7 or 8 having substantially the same promoter activity as the promoter of the ϵ -cyclases as shown in SEQ ID NO: 1, 7 or 8 and

iii) functionally equivalent fragments of the sequences under
1) or ii) having substantially the same promoter activity
as the promoter of ϵ -cyclases as shown in SEQ ID NO: 1, 7
or 8.

- 10 3. A method for identifying and/or isolating promoters of genes
which code for an ϵ -cyclase, where at least one nucleic acid
sequence or a part thereof is employed in the identification
and/or isolation, where said nucleic acid sequence codes for
an amino acid sequence which comprises at least one sequence
as shown in SEQ ID NO: 17, 18, 19, 20, 21 or 22 or a
variation of these sequences.
4. The method as claimed in claim 3, where said nucleic acid
sequence comprises a sequence as shown in SEQ ID NO: 23, 25,
27, 29, 31, 33, 35, 37, 39, 41, 43 or 45.
5. The method as claimed in either of claims 3 or 4, where the
method is carried out with use of the polymerase chain
reaction, and said nucleic acid sequence or a part thereof is
employed as primer.
- 20 6. A method for producing a transgenic expression cassette with
specificity for the flower of plants, including the following
steps:
- I. isolation of a promoter sequence, where at least one
nucleic acid sequence or a part thereof is employed in
the isolation, where said nucleic acid sequence codes for
an amino acid sequence which comprises at least one
sequence as shown in SEQ ID NO: 17, 18, 19, 20, 21 or 22
or a variation of these sequence motifs.
- 30 II. functional linkage of said promoter sequence to a further
nucleic acid sequence, where said nucleic acid sequence
is heterologous in relation to the promoter.
7. The method as claimed in claim 6, where said nucleic acid
sequence includes a sequence as shown in SEQ ID NO: 23, 25,
27, 29, 29, 31, 33, 35, 37, 39, 41, 43 or 45.
8. The method as claimed in either of claims 6 or 7, where the
method is carried out with use of the polymerase chain
reaction, and said nucleic acid sequence or a part thereof is
employed as primer.

9. A transgenic expression cassette for the targeted transgenic expression of nucleic acid sequences in the flower of plants, including
- a) at least one promoter sequence of a gene coding for an ϵ -cyclase, and
 - b) at least one further nucleic acid sequence, and
 - c) where appropriate further genetic control elements,
- where at least one promoter sequence and one further nucleic acid sequence are functionally linked together, and the
- 10 further nucleic acid sequence is heterologous in relation to the promoter sequence.
10. The transgenic expression cassette as claimed in claim 9, where the promoter sequence of a gene coding for an ϵ -cyclase is a sequence selected from the group of sequences consisting of
- i) the promoter sequence of the ϵ -cyclase from *Tagetes erecta* as shown in SEQ ID NO: 1, the ϵ -cyclase from *Arabidopsis thaliana* as shown in SEQ ID NO: 7, the ϵ -cyclase from *Oryza sativa* as shown in SEQ ID NO: 8, and
 - 20 ii) functional equivalents of the promoter sequences as shown in SEQ ID NO: 1, 7 or 8 having substantially the same promoter activity as the promoter of the ϵ -cyclases as shown in SEQ ID NO: 1, 7 or 8 and
 - iii) functionally equivalent fragments of the sequences under i) or ii) having substantially the same promoter activity as the promoter of ϵ -cyclases as shown in SEQ ID NO: 1, 7 or 8.
11. The transgenic expression cassette as claimed in claim 9 or 10, where the nucleic acid sequence to be expressed
- 30 transgenically enables
- a) the expression of a protein encoded by said nucleic acid sequence, or
 - b) the expression of a sense RNA, antisense RNA or double-stranded RNA encoded by said nucleic acid sequence.

12. An isolated nucleic acid sequence comprising
- a) the *Tagetes erecta* ϵ -cyclase promoter as shown in SEQ ID NO: 1 or
 - b) a functionally equivalent fragment of a) with substantially the same promoter activity as a).
- 10 13. The isolated nucleic acid sequence as claimed in claim 12, including, in the 3' orientation to the *Tagetes erecta* ϵ -cyclase promoter as shown in SEQ ID NO: 1 or a functionally equivalent fragment of the aforementioned, a sequence coding for a 5'-untranslated region and/or a transit peptide.
14. The isolated nucleic acid sequence as claimed in claim 12 or 13 including a sequence described by SEQ ID NO: 2 or 3.
15. A double-stranded RNA molecule comprising
- a) a sense RNA strand comprising at least one ribonucleotide sequence which is substantially identical to at least part of a nucleic acid sequence coding for the promoter region of an ϵ -cyclase, and
 - 20 b) an antisense RNA strand which is substantially complementary to the RNA sense strand under a).
16. The double-stranded RNA molecule as claimed in claim 15, where the promoter region of the ϵ -cyclase comprises a sequence selected from the sequences as shown in SEQ ID NO: 1, 7 or 8.
17. A ribonucleic acid molecule comprising
- a) at least one ribonucleotide sequence which is substantially identical to at least one part of a nucleic acid sequence coding for the promoter region of an ϵ -cyclase, and
 - 30 b) at least one further ribonucleotide sequence which is substantially complementary to at least one part of the ribonucleotide sequence under a),

where a) and b) are connected together covalently, and further functional elements may be located where appropriate between a) and b).

18. The ribonucleic acid molecule as claimed in claim 17, where the promoter region of the δ -cyclase includes a sequence selected from the sequences as shown in SEQ ID NO: 1, 7 or 8.

19. A transgenic expression cassette, comprising

a) at least one promoter functional in plants, and

10 b) at least one nucleic acid sequence coding for a double-stranded RNA molecule as claimed in either of claims 15 or 16 or coding for a ribonucleic acid molecule as claimed in either of claims 17 or 18,

where at least one of said promoters and at least one of said nucleic acid sequences are functionally linked together, and the promoter is heterologous in relation to the nucleic acid sequence.

20. The transgenic expression cassette as claimed in claim 19, where the promoter is a promoter having specificity for the flower of plants.

20 21. A transgenic expression vector comprising a nucleic acid sequence as claimed in any of claims 12 to 14 or a transgenic expression cassette as claimed in any of claims 9, 10, 11, 19 or 20.

22. A transgenic organism comprising a nucleic acid sequence as claimed in any of claims 12 to 14, a double-stranded RNA as claimed in claim 15 or 16, a ribonucleotide sequence as claimed in claim 17 or 18, a transgenic expression cassette as claimed in any of claims 9, 10, 11, 19 or 20 or a transgenic expression vector as claimed in claim 21.

30 23. The transgenic organism as claimed in claim 22 selected from the group consisting of bacteria, yeasts, fungi, animal and plant organisms.

24. The transgenic organism as claimed in claim 22 selected from the group consisting of bacteria, yeasts, fungi, non-human animal and plant organisms or cells, cell cultures, parts, tissues, organs or propagation material derived therefrom.

25. The transgenic organism as claimed in claim 23 or 24 selected from the group of agricultural crop plants.
26. The use of an isolated nucleic acid sequence as claimed in any of claims 12 to 14, of a double-stranded RNA as claimed in claim 15 or 16, of a ribonucleotide sequence as claimed in claim 17 or 18, of a transgenic expression cassette as claimed in any of claims 9, 10, 11, 19 or 20, of a transgenic expression vector as claimed in claim 21 of a transgenic organism as claimed in any of claims 23 to 25 or cell cultures, parts, organs, tissues or transgenic propagation material derived therefrom in methods for the transgenic expression of nucleic acids or proteins.
27. The use of an isolated nucleic acid sequence as claimed in any of claims 12 to 14, of a double-stranded RNA as claimed in claim 15 or 16, of a ribonucleotide sequence as claimed in claim 17 or 18, of a transgenic expression cassette as claimed in any of claims 9, 10, 11, 19 or 20, of a transgenic expression vector as claimed in claim 21, or of a transgenic organism as claimed in any of claims 23 to 25 or cell cultures, parts, organs, tissues or transgenic propagation material derived therefrom for producing human or animal foods, seeds, pharmaceuticals or fine chemicals.
28. A method for producing human or animal foods, seeds, pharmaceuticals or fine chemicals, where a transgenic organism as claimed in any of claims 23 to 25 is cultured, and the desired human or animal foods, seeds, pharmaceuticals or fine chemical is produced and/or isolated using said organism.
29. A method for producing ketocarotenoids, where the mRNA amount and/or activity of at least one β -cyclase is reduced by introducing at least one double-stranded RNA as claimed in claim 15 or 16, one ribonucleotide sequence as claimed in claim 17 or 18 or one transgenic expression cassette as claimed in either of claims 19 or 20.

Application number/numéro de demande: EP03/08394

Figures: 1

Pages: _____

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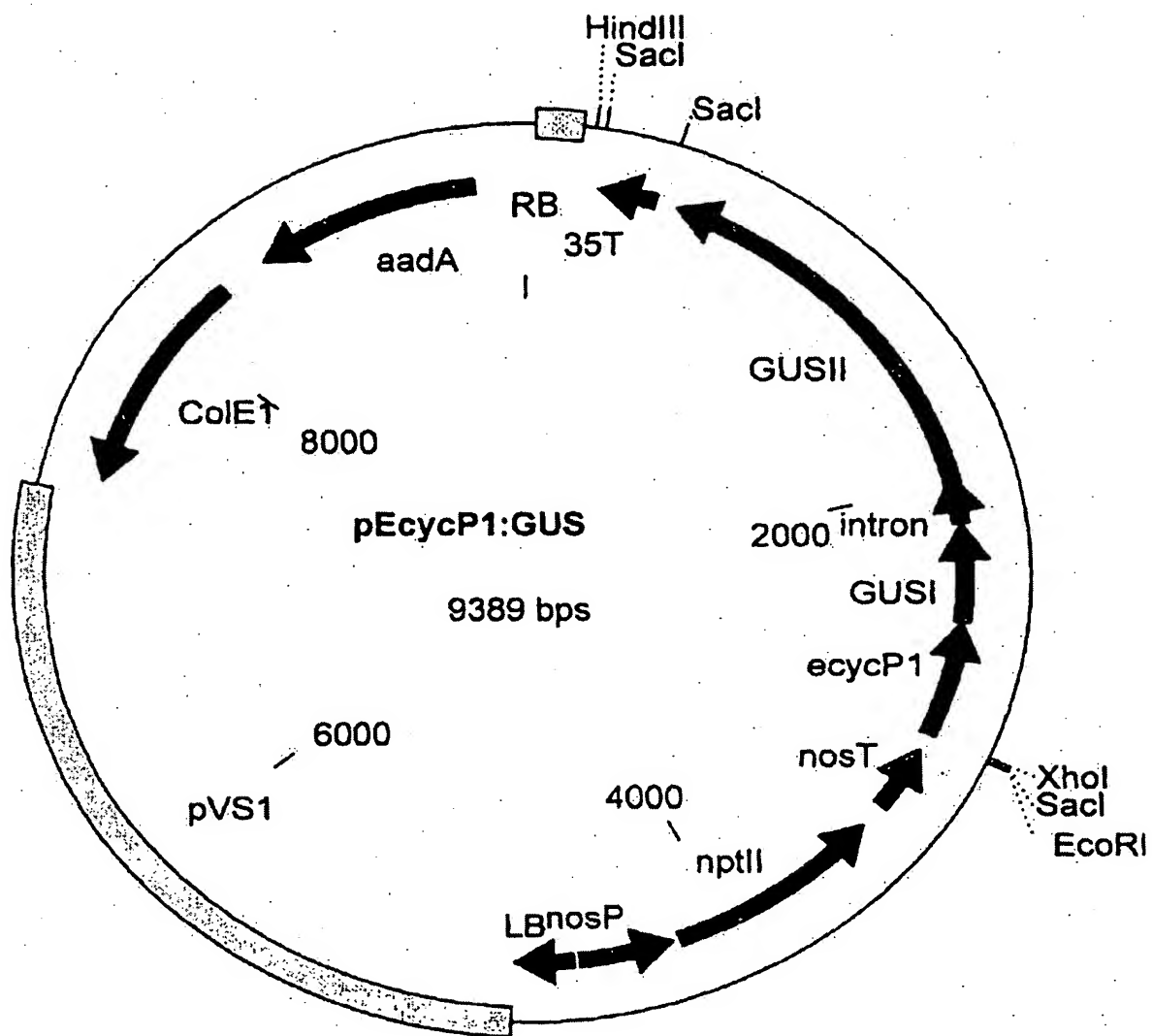


Fig.2

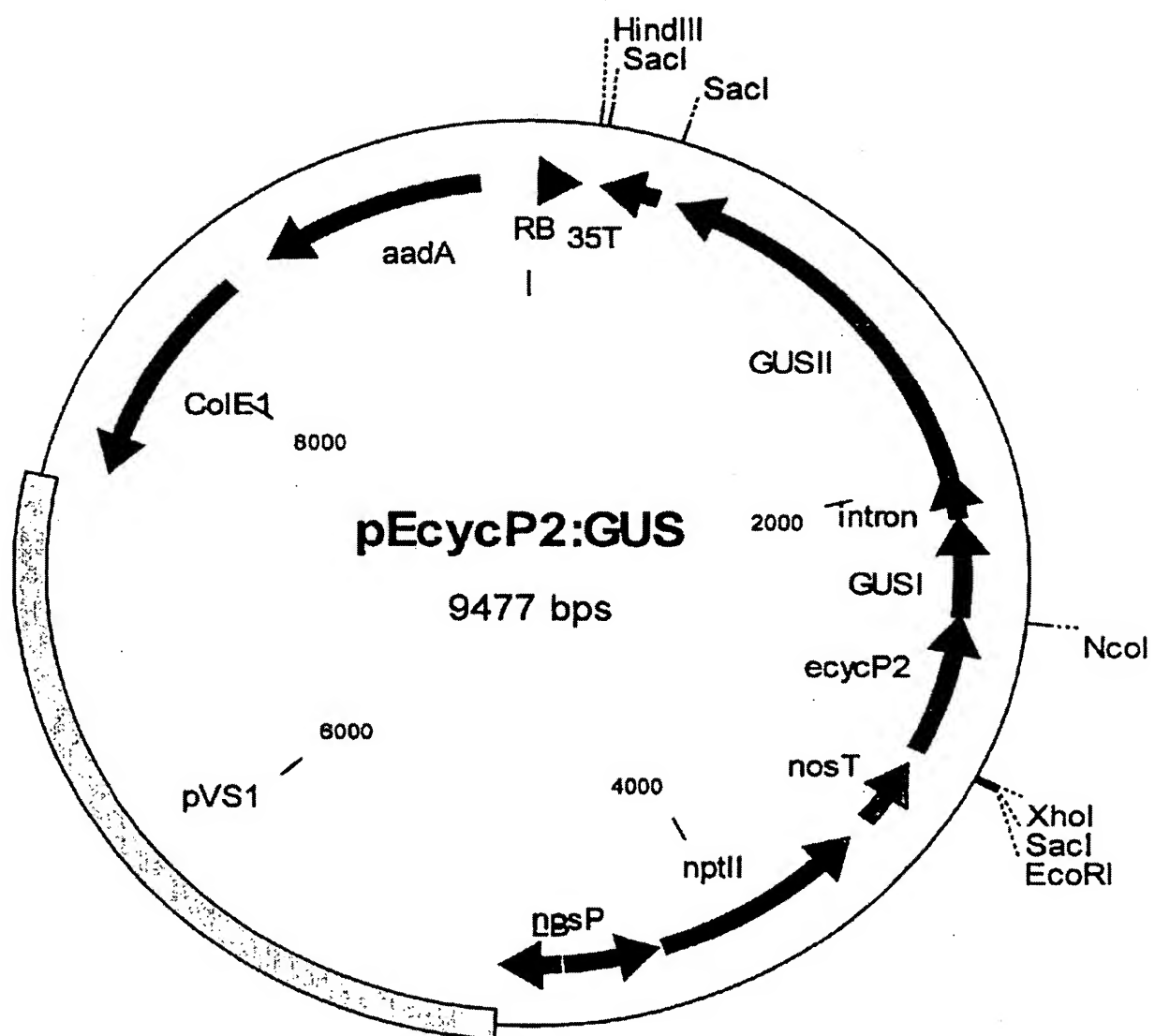


Fig.3

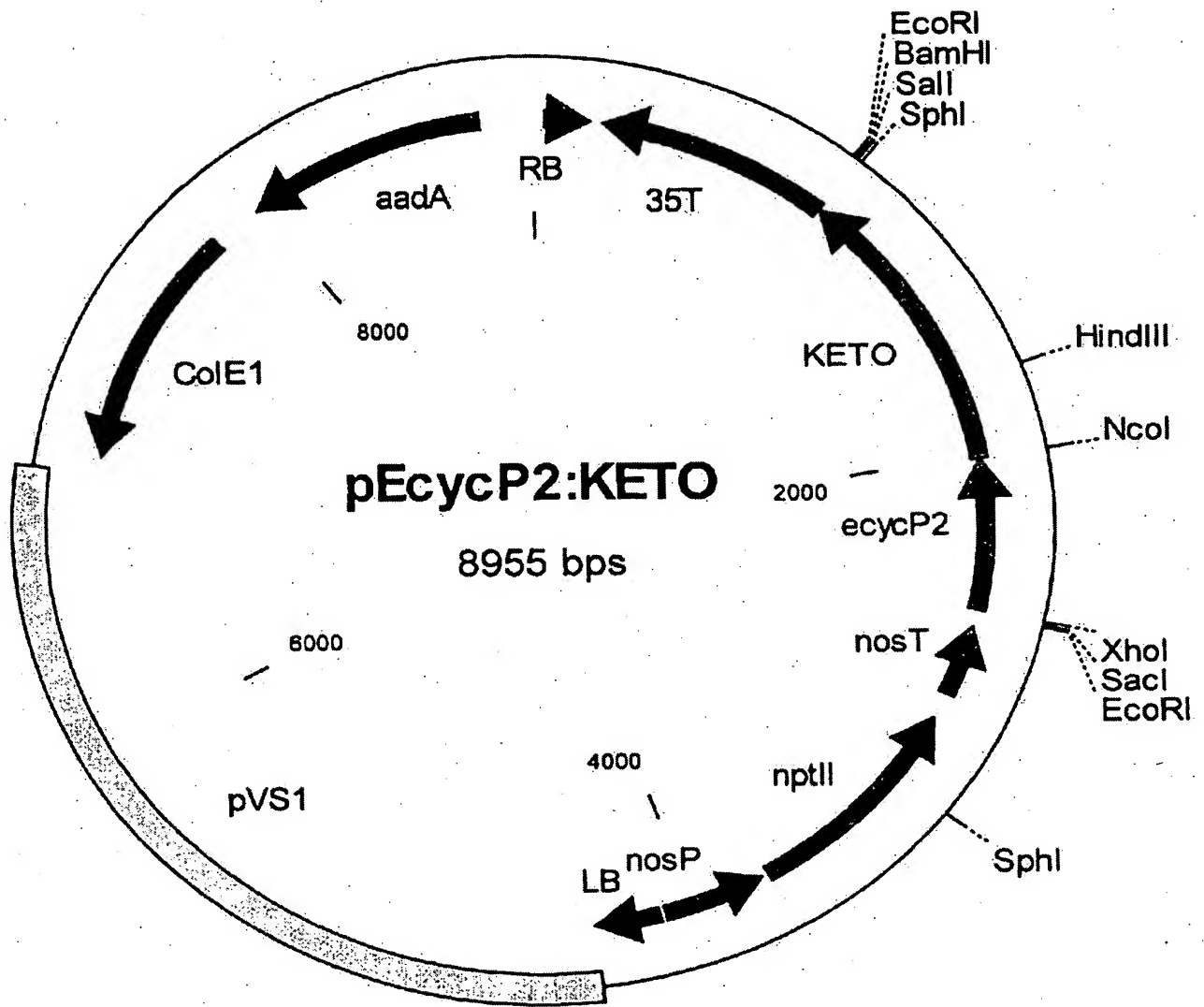


Fig.4

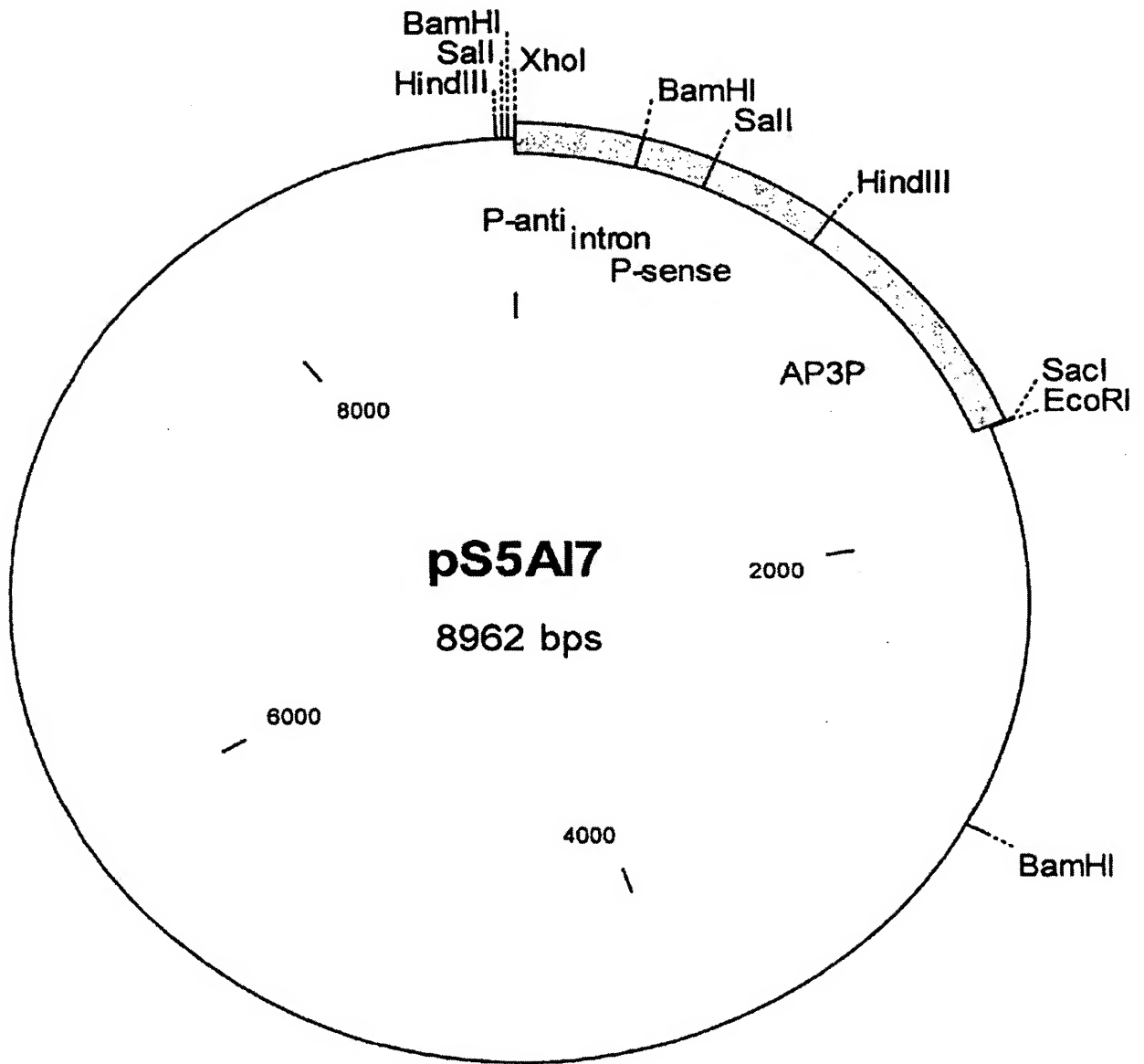


Fig.5

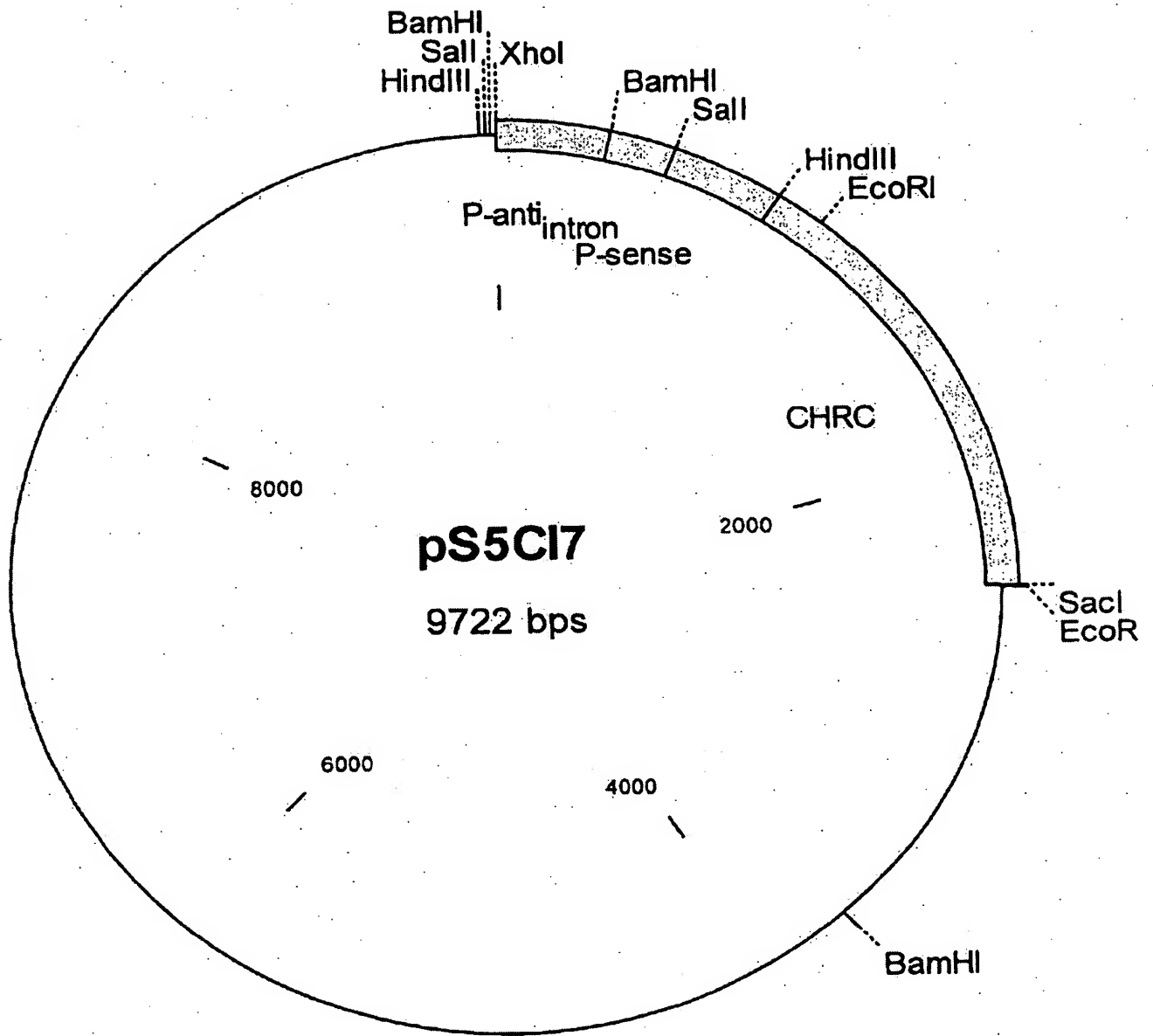


Fig.6

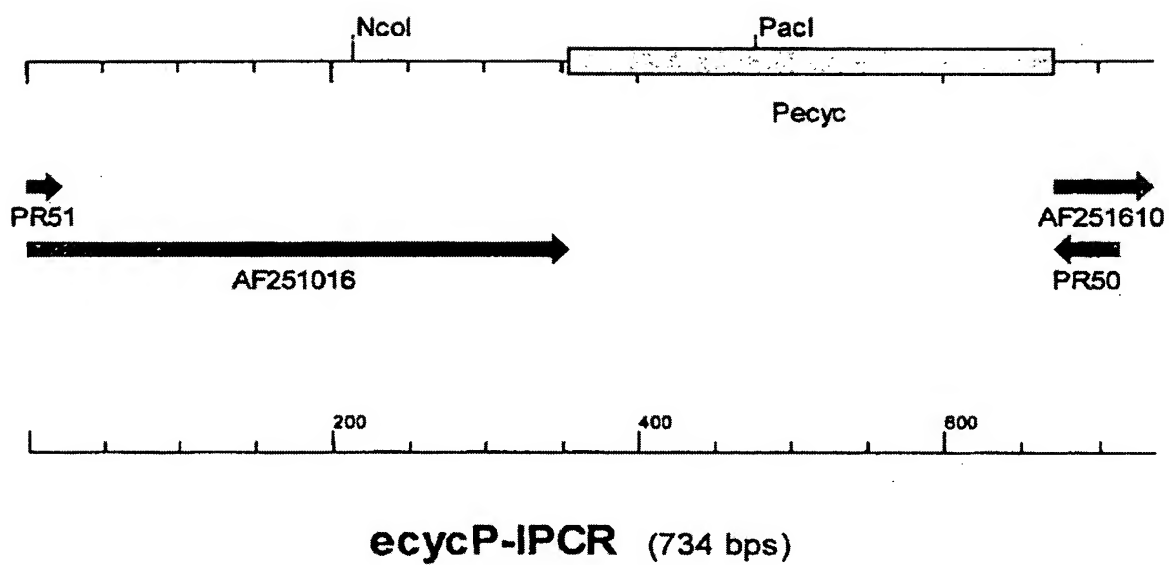


Fig.7

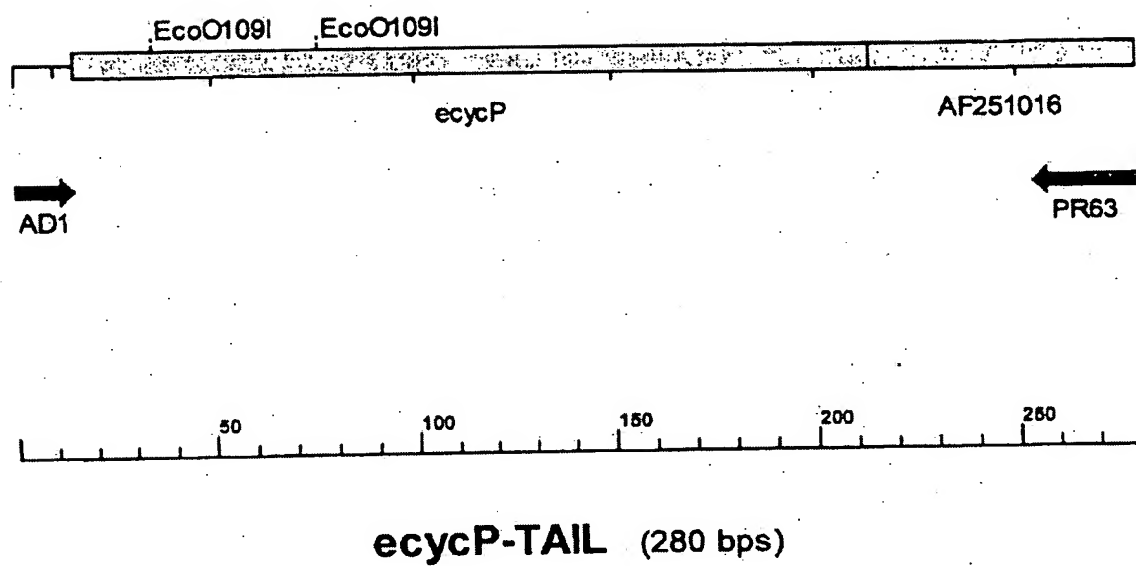


Fig.8

Fig. 9

KETO2.pro	MQLAATVMLEQLTGSAEALKEKEKEVAGSSDVLRTWATQYSLPSEESDAA	50
X86782.pro	MQLAATVMLEQLTGSAEALKEKEKEVAGSSDVLRTWATQYSLPSEESDAA	50
KETO2.pro	RPGLKNAYKPPPSDTKGITMALAVIGSWAAVFLHAI FQIKLPTSLDQLHW	100
X86782.pro	RPGLKNAYKPPPSDTKGITMALRVIGSWAAVFLHAI FQIKLPTSLDQLHW	100
KETO2.pro	LPVSDATAQLVSGSSSLLHI VVVFVLEFLYTGLFITTHDAMHGTI AMRN	150
X86782.pro	LPVSDATAQLVSGTSSLLDI VVVFVLEFLYTGLFITTHDAMHGTI AMRN	150
KETO2.pro	RQLNDFLGRVCI SLYAWFDYNMLHRKHWEEHHNHTGEVGKDDPFHRGNPGI	200
X86782.pro	RQLNDFLGRVCI SLYAWFDYNMLHRKHWEEHHNHTGEVGKDDPFHRGNPGI	200
KETO2.pro	VPWFASFMSMYMSMWQFARLAWWT VVMQLLGAPMANLLVFMAAAPILSAF	250
X86782.pro	VPWFASFMSMYMSMWQFARLAWWT VVMQLLGAPMANLLVFMAAAPILSAF	250
KETO2.pro	RLFYFGTYMPHKPEPGAA SSGSSPA VMNWWKSRTSQASDLVSFLT CYHFDL	300
X86782.pro	RLFYFGTYMPHKPEPGAA SSGSSPA VMNWWKSRTSQASDLVSFLT CYHFDL	300
KETO2.pro	HWEHHRWPFAPWWELPNCRRLSGRGLVPA	329
X86782.pro	HWEHHRWPFAPWWELPNCRRLSGRGLVPA	329

Fig.10

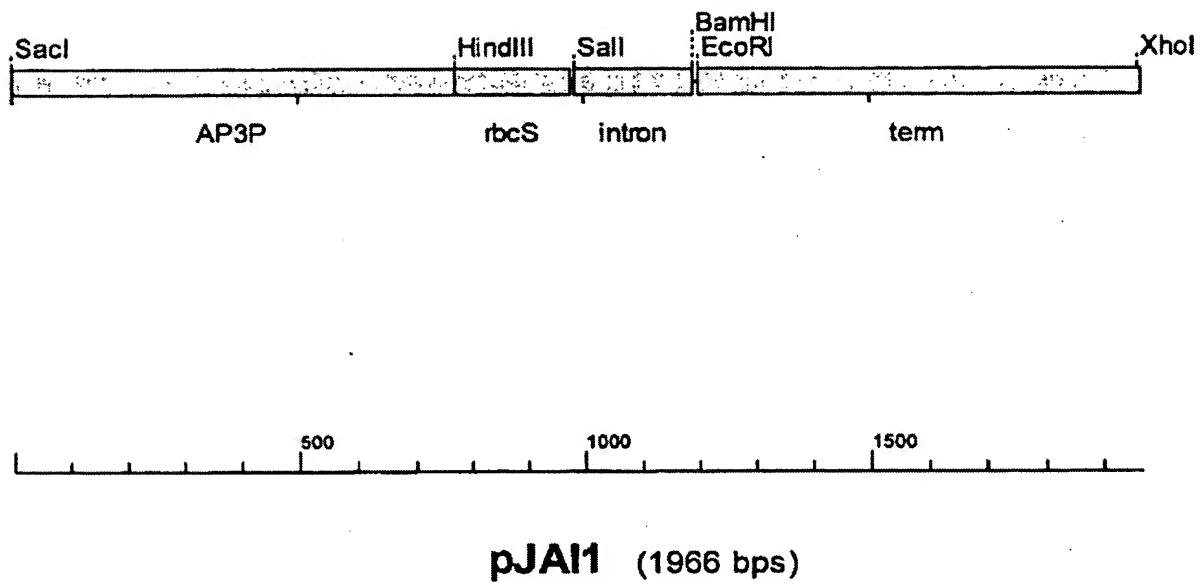


Fig.11

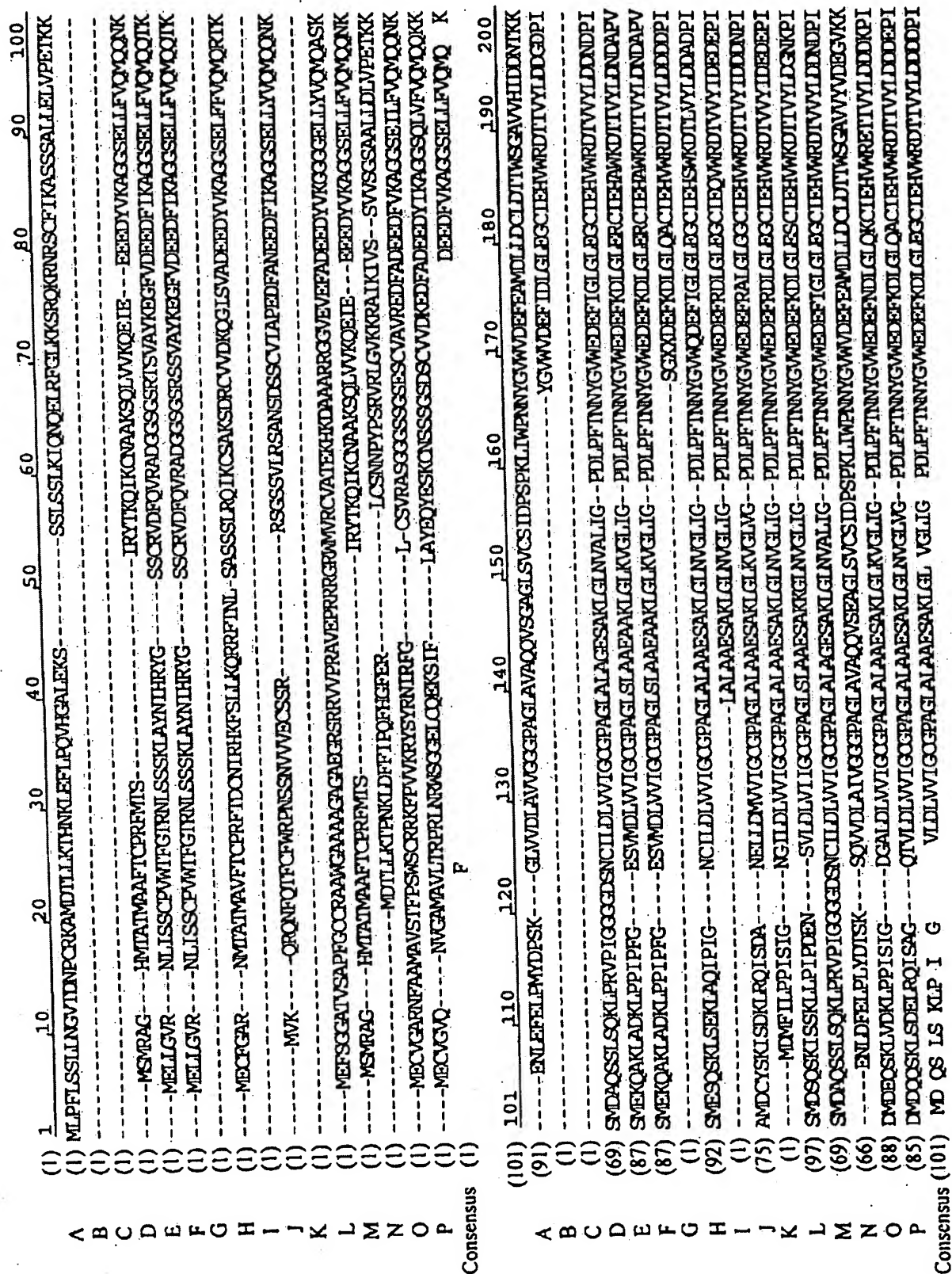


Fig. 12A

	201	210	220	230	240	250	260	270	280	290	300
A	(201)	DLNRPYGRVNRKLLKSKMLQKCTINGVKFQAKVIKVIHEE--SKSILLIENDGVYTIQAUVLDATGFSRCLVQIDKPNP--GYQVAYGILAEVEEQPDL									
B	(182)	MIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
C	(35)	(1) -IGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
D	(167)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
E	(180)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
F	(180)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
G	(34)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
H	(185)	RIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
I	(61)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
J	(168)	YIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
K	(88)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
L	(190)	MIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
M	(167)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
N	(157)	DLSPYGRVNRKLLKSKMLQKCTINGVKFQAKVIKVIHEE--ANSTVWSDGVKIQASVLDATGFSRCLVQIDKPNP--GYQVAYGILAEVEEQPDL									
O	(181)	TIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
P	(178)	LIGRAYGRVSRHLLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
Consensus	(201)	LIGRAYGRVSR LLHEELLKRCVSGSVYSSKVEKIEAGDGHSLVECENNIVIPORLAVASGAASEKLLQYEVGGPRVSVQIYAGVEVEVENNPYP									
A	(279)	DRMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
B	(135)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
C	(100)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
D	(267)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
E	(280)	NLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
F	(280)	NLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
G	(134)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
H	(285)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
I	(161)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
J	(268)	NLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
K	(188)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
L	(290)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
M	(267)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
N	(254)	DRMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
O	(281)	DQMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
P	(278)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									
Consensus	(301)	SLMVFMDYRDYTKVPGME---AEYPTFLYAMP-----									

Fig.12B

(401) 401 410 420 430 440 450 460 470 480 490 500
 (370) VLPQVVGIGGTAGMHPSTGMMVARTLAAPIVANAIVRSLS--D-----RSLSGKLSAEWKDLWPIERRQREFFCFGMDILLKLLPATRRFF
 (166) -----
 (188) NTEQKNLAFGAAR-----
 (355) NTEQKNLAFGAASWHPATGYSVRSLSAENVAAVIAKILGKNSKQMLDHERVTN-ISKQWETLWPIERKQRAFFLFGIALIVQMDIEGIRTF
 (368) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASVIAKILKQNSAVWSQSSAVN-ISKQWSSLWPKERKQRAFFLFGIALIVQMDIEGIRTF
 (368) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASVIAKILKQNSAVWSQSSAVN-ISKQWSSLWPKERKQRAFFLFGIALIVQMDIEGIRTF
 (222) NTEQKNLAFGAASWHPATGYSVRSLSAEPKCAFVIANILKQNSKMLTSS-STPS-ISTQWNVILWPKERKQRSFFLFGIALILQIDIEGIRSF
 (373) NTEQKNLAFGAASWHPATGYSVRSLSAENVAAVIAKILQDQSKEMISLGKYN--ISKQWETLWPIERKQRAFFLFGIALIVQMDIEGIRTF
 (249) NTEQKNLAFGAAS-----
 (356) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASAIAANILKNDLSKVALRORSVGN-ISKQWNVILWPKERKQRAFFLFGIALIVQMDIEGIRTF
 (276) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASAIAVILKDHSGRLTHEQENEN-ISKQWNVILWPKERKQRAFFLFGIALILQIDIEGIRTF
 (378) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASVSDILRNVPGEVLPGTSQSSPSMLAMKILWPKERKQRSFFLFGIALILQIDIEGIRTF
 (364) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASVIAKILGKNSKQMLDGLRYTN-ISKQWETLWPIERKQRAFFLFGIALIVQMDIEGIRTF
 (345) VLPQVVGIGGTAGMHPSTGMMVARTLAAPIVANAIVRSLSP-SS-----NSLRQQLSAEWRDLWPIERRQREFFCFGMDILLKLLPATRRFF
 (369) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASVIAEILRETT-----KQINEN-ISRQWNTLWPKERKQRAFFLFGIALIVQMDIEGIRSF
 (166) NTEQKNLAFGAASWHPATGYSVRSLSAEPKVASVIANILRQHSKMLTSS-SIPS-ISTQWNVILWPKERKQRSFFLFGIALILQIDIEGIRSF
 Consensus (401) NTEQKNLAFGAASWHPATGYSVRSLSAEP YASVIA ILR S L IS QAW TLWP ERKQRAFFLFGIALIVQMDIEG RTFF

(501) 501 510 520 530 540 550 563
 (462) DAFDLEPRYMHGFTSSRLFLPELLVFGLSLFSHASNTSRLEKIMAKGILPLVNMNNLVQDID
 (166) -----
 (202) -----
 (454) RTFFRLPTWMMGFTGSSLSSTDLIIFAFYMFIIAPHSLRMGLVRHLSDPTGDMKAYLTI
 (467) RTFFRLPTWMMGFTGSSLSSTDLVLFMYMEVLAPNSVRMSLVRHLSDPSGAMWKAYLER
 (467) RTFFRLPTWMMGFTGSSLSSTDLVLFMYMEVLAPNSVRMSLVRHLSDPSGAMWKAYLER
 (320) RAFFRVPKWMAQCGFTGSSLSXADIMLFAFYMFIIAPNDMRRGLIRHLSDPTGATLIRTYLTF
 (471) RTFFRLPKWMMGFTGSSLSSTDLIIFALYMEVIAPHSLRMGLVRHLSDPTGATMWKAYLTI
 (263) -----
 (455) RTFFRVPKWMMGFTGSSLSADILIFAFYMEFFIAPNDLPMGLIRHLSDPTGATMIRTYTIL
 (375) RTFFRLPKWMMHGFGLSSLSADILIFAFYMFIIAPNDLRKCLIRHLVSDPTGATMRTYTLIL
 (478) RTFFRLPKWMMHGFGLSSVVDLILFAFYMFIIAPNQVRMLVRHLSDPTGSTMKTLYLIL
 (463) RTFFRLPTWMMGFTGSSLSSTDLIIFAFYMFIIAPHSLRMGLVRHLSDPTGDMKAYLTI
 (439) DAFDQLPHYMHGFTSSRLFLPELLVFGLSLFSHASNTSRLEIMTKGIVPLAKMNNLVQDID
 (462) RTFFRLPKWMAQCGFTGSSLSSTDLVLFALYMEVISPNLRKGLIRHLSDPTGATMKTLYLV
 (464) RAFFRVPKWMAQCGFTGSSLSADIMLFAFYMFIIAPNDMRRGLIRHLSDPTGATLIRTYLTF
 Consensus (501) RTFFRLP MMW GFTGSSLS DLIIFA YMFIIAPN LPM LVRHLSDPTGATMUK YLTI

Fig.12C

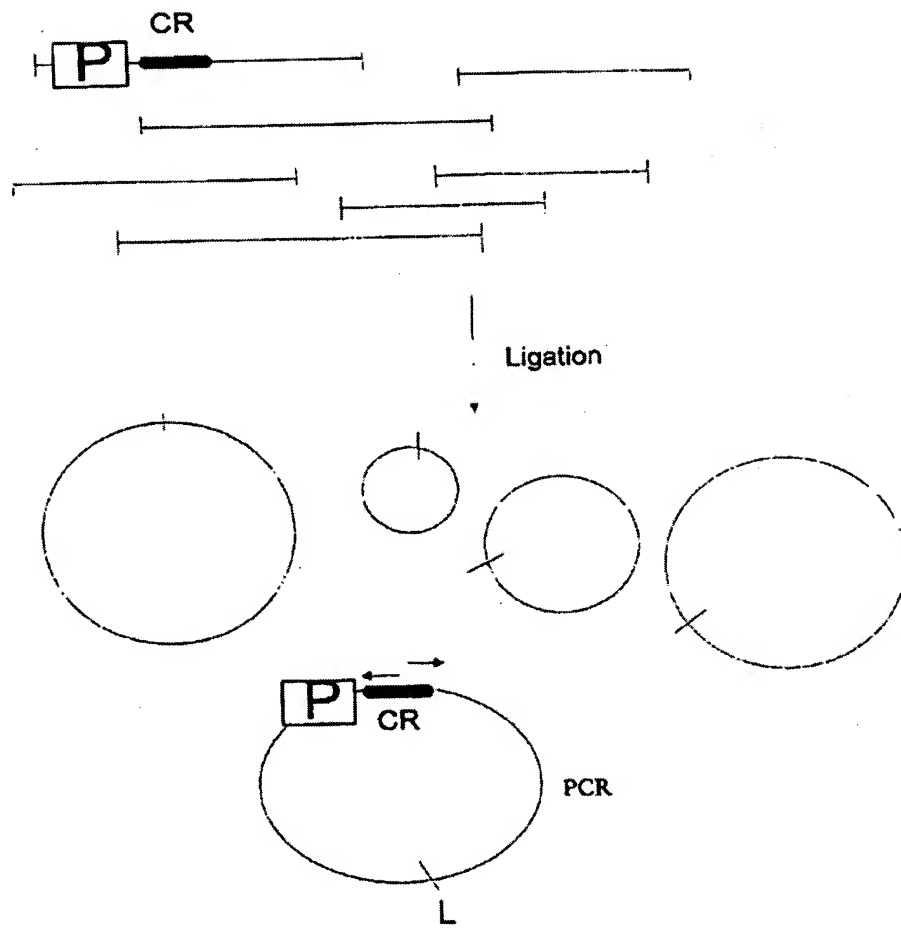


Fig. 13

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